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"BOTHRIOCEPHALUS ANEMIA"

Diphyllobothrium latum and Pernicious Anemia[1]

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[1] Submitted for publication November 6, 1930.

INTRODUCTION

More than 250 cases of infestation of human beings with *Diphyllobothrium latum* have been reported on the continent of North America, since Walker (455) and Leidy, in 1879, announced the first case noted in an American.

The first case of native infestation was recorded in 1901, in a French-Canadian who never had been outside of Canada, and only rarely outside of the province of Quebec (172). Altogether, there are on record at the present time twenty-three certain cases and one questionable case of native infestation (table 1). The remainder of the cases are in immigrants, mainly from Baltic states (tables 2 and 3).

The frequency of infestation is apparently increasing (19, 463, 464). The fish tapeworm is now estimated to be the most common of cestodes in the North-Central states and central Canada (348). Furthermore, in one large pathologic laboratory in New York City it was identified in more than 50 per cent of infestations with tapeworm (112). Pilot found the same predominance in Chicago, and Barron in Minneapolis during the years 1911 to 1929 saw in his private practice nineteen cases of infestation with Diphyllobothrium against two of infestation with *Taenia saginata.*

Endemic infestation of fresh water fish with plerocercoids of this cestode has been definitely proved in the lake region of northern Minnesota, central and northern Canada and the Portage Lake area in Michigan, (125, 258, 260, 445–449, 460, 461, 463, 464), the degree of infestation in the various lakes and rivers depending largely on the racial origin of the population living at or near the waterfront. In this respect, Finlanders and Russians (Jews) are of greatest significance. Immigrants from the Baltic states are responsible for the establishment of this infestation; to the upkeep and dissemination of it the great number of dogs owned by the white and Indian population aids materially, and fish-eating wild animals (wolf, coyote, fox, bear and lynx) may assist in this spread (447, 459, 463). A further increase may therefore be expected as long as fish with living plerocercoids are being consumed.

Diphyllobothrium, even in large numbers, may be harbored for decades without causing any ill effects in the host, and the percentage in which it gives rise to clinical symptoms is small. Thus, one may assume that infestation on this continent is considerably greater than is indicated by the published reports, since few cases find their way into the literature unless they present unusual features.

Great practical and theoretical interest is attached to the fact that more than thirty-five of American and Canadian patients, about 14 per cent, have been reported as being anemic, most of them presenting objective evidence to that effect (table 3). If with Ehrström (118) it is assumed that on the average anemia develops in only one of 5,000 to 10,000 carriers as a result of the parasitism, then an even stronger indication of the wide extent of this infestation exists. Attention

TABLE 1

Cases of infestation with Diphyllobothrium latum

CASES	YEAR REPORTED	INVESTIGATOR	STATE OR PROVINCE	PLACE OF BIRTH AND RACE
		Natives of the United States		
1*	1903	Bellows	Pennsylvania	Pennsylvania
1†	1906	Nickerson (303)	Minnesota	Minnesota (Finnish)
1	1911	Nickerson (304)	Minnesota	Minnesota
1	1912	Warthin (463)	Michigan	Michigan
1	1919	Riley (347)	Minnesota	Minnesota
1	1919	Riley	Minnesota	Chippewa Indian
2	1922	Calvin	Illinois	Illinois
1	1922	Wallace and Grant	Indiana	Indiana
1	1926	Levy and Pierson	Michigan	Michigan
1	1926	Lyon (255)	Indiana	Indiana
2	1928	McGavran and Songkla	Massachusetts	Massachusetts (Jew)
1‡	1928	Warthin (464)	Michigan	Michigan (Jew)
1	1928	Warthin	Michigan (?)	Native (Jew)
1	1928	Vergeer (446)	Wisconsin	Wisconsin
1	1928	Pilot	Illinois	Illinois (Jew)
3	1929	Barron	Minnesota	Minnesota (?)
2	1929	Wilder and Rodda	Minnesota	Minnesota
		Native Canadians		
1	1901	Hamilton	Quebec	Quebec
1	1928	Nicholson	Manitoba	Manitoba

* Questionable.

† Anemic.

‡ Mild secondary anemia.

TABLE 2

Cases of infestation with Diphyllobothrium latum, without anemia

CASES	YEAR REPORTED	INVESTIGATOR	STATE OR PROVINCE	PLACE OF BIRTH AND RACE
Immigrants in the United States				
1	1879	Walker (455), Leidy	Pennsylvania	Sweden
1	1891	Packard	Pennsylvania	Finland
1	1898	Dock	Colorado	Europe
2	1898	Dock	Texas	Europe
1	1901–02	Willson, R. N. (474, 475)	Pennsylvania	?
1	1902	Riesman	Pennsylvania	Russia
1	1902	Stengel (418)	Pennsylvania	Europe
1	1902	Stengel	Pennsylvania	Scandinavia
1	1902	McFarland	Pennsylvania	Russia (Jew)
1	1903	Janeway	New York	Finland
1	1903	Ewing	New York	Sweden
1	1903	Hastings	New York	Europe
4	1903	Berkeley	New York	Russia
1	1904	Coplin	New York	Finland
1	1904	Walker, J. M. (454)	Colorado	Sweden
1	1904	Edsall (114)	Pennsylvania	Finland
2	1904	Edsall (113)	Massachusetts	Russia
1	1905	Hygienic Laboratory (191)	Washington, D. C.	Russia
2	1906	Nickerson (303)	Minnesota	Finland
1	1907	Van Nuys	Ohio	Baltic States
6	1908	Huber (187)	New York	Poland
1	1908	Boswell (54)	New York	Russia
1	1908	Wilson, R. L. (476)	Louisiana	Germany
60	1911	Nickerson (304)	Minnesota (49)	Sweden (2) Japan (1)
		Nickerson	U. S. A. (11)	Finland (57)
50*	1912	Warthin (463)	Michigan	Europe (mostly Finlanders)
1	1916	Kopelowitz	Missouri	Russia
1	1916	Rubenstone	New York	Russia
1	1916	Singer	Missouri	Russia
7†	1916	Becker (33)	Illinois	Russia or Germany
1	1919	Riley (346)	Minnesota	Russia
1	1919	Riley	Minnesota	Sweden
1	1920	Lyon (254)		Russia
1	1924	Anderson	New Mexico	Sweden
1	1927	Todd		?
1	1928	Vergeer (414)	Michigan	Germany
1	1928	Pilot	Illinois	Russia (Jew)
1	1929	Magath (260)	Illinois	Poland

TABLE 2—*Concluded*

CASES	YEAR REPORTED	INVESTIGATOR	STATE OR PROVINCE	PLACE OF BIRTH AND RACE
Immigrants in the United States—*Concluded*				
1	1929	Magath	Illinois	Lithuania
2	1929	Magath	Illinois	Russia
1	1929	Magath	Minnesota	Russia
1	1929	Magath	Minnesota	Finland
1	1929	Magath	Minnesota	Roumania
14	1929	Barron	Minnesota	?
Immigrants in Canada				
12	1928	Nicholson	Manitoba	?
3	1929	Magath (260)	Manitoba	Russia
1	1929	Magath	Manitoba	Austria
1	1929	Magath	Ontario	Poland

* Majority observed to appear more or less anemic.
† One case possibly native.

should be called to the absence of anemia in all infested natives of the United States and Canada (table 1). Most patients with anemia are from countries that form the eastern shore of the Baltic Sea.

The anemia associated with *Diphyllobothrium latum* has been the subject of intensive investigation in Finland in the last half century. The two outstanding contributions to knowledge of this phenomenon are the two monographs of Schauman (381), the first one published in 1894, the last one in 1925 in collaboration with Saltzman (389). Their material deals with 326 cases of this kind, the majority of which were personally observed. Schauman had made pernicious anemia and especially Diphyllobothrium anemia a life study and is recognized as an authority in this field.

Exhaustive research and studies of cases have led to the assumption of the identity of Diphyllobothrium anemia and pernicious anemia, a viewpoint which at the present time is shared by the majority of writers. Now and then, however, doubt is voiced against this assumption, and although the issue seems well settled abroad, on this side of the ocean hesitancy is felt in accepting Schauman's viewpoint. Most of the cases reported on this continent have been more or less sporadic and casual and have not contributed much to the discussion of this

TABLE 3

Cases of infestation with Diphyllobothrium latum with anemia

CASES	YEAR REPORTED	INVESTIGATOR	STATE OR PROVINCE	PLACE OF BIRTH AND RACE	ANEMIA, GRADE*
Immigrants into United States					
1	1896	Hagelstam	New York	Finland	2
1	1896	Hagelstam	Michigan	Finland	2
1	1904	Edsall (114)	Pennsylvania	Finland	3
1	1905	Thompson	New York	Finland	3
1	1905	Meyer (272)	New York	Finland	3
1	1905	Meyer (272)	New York	Poland	3
1	1906	Nickerson (303)	Minnesota	Finland	3
1	1907	Lynah	New York	Sweden	2
1	1908	Huber (186)	New York	Poland	2
1	1912	Warthin (463)	Michigan	Finland	Severe
1	1915	Cabot (75)	?	Finland	3
1	1916	Schmidt	Michigan	Finland	3
1	1917	Levy and Wall		Finland	1
1	1917	McGaffin		Finland	3
3	1917	Martin	Minnesota	?	3
4	1920	Minot	United States	?	Present
1	1920	Savitz	Pennsylvania	Finland	Secondary
1	1923	Haden	Michigan	Finland	2
1	1923–24	Riley (348)	Minnesota	Russia	1
1	1924	Cohen	New York	Finland	3
1	1926	Rosenthal	New York	Austria	Moderate secondary
1	1928	Warthin (464)	Michigan	Finland	Marked (died)
1	1928	Warthin (464)	Michigan	? (Jew)	Severe secondary
1	1928	Isaacs, Sturgis and Smith	Michigan	Lithuania	3
1	1928	Richter, Maurer and Eyl	?	Finland	3
1	1929	Barron	Minnesota	Finland	4
†	1928	Kerr and Falconer	United States	?	Present
Immigrants into Canada					
1	1928	Nicholson	Manitoba	Russia	1
1	1928	Nicholson	Manitoba	Russia (Jew)	2
1	1928	Nicholson	Manitoba	Finland	Aplastic
1	1928	Cammeron and Foster		?	3

* Graded on the basis of 1 to 4: 1 = 4,000,000 to 3,000,000 erythrocytes for each cubic millimeter of blood; 2 = 3,000,000 to 2,000,000 erythrocytes for each cubic millimeter of blood; 3 = 2,000,000 to 1,000,000 erythrocytes for each cubic millimeter of blood; 4 = below 1,000,000 erythrocytes for each cubic millimeter of blood.

† Number of cases not given. One case was recorded by Levine and Ladd the reference to which was found too late to be included in the table.

type of anemia. It would indeed be very desirable to have every case investigated as thoroughly as has been done in the case reported by Isaacs, Sturgis and Smith. Special emphasis should be placed on the more neglected signs: glossitis; achylia; pathologic changes in the spinal cord; volume index; diameter of the erythrocytes; the reticulocyte response to treatment with liver; leukocyte, platelet and differential counts, and morphologic studies of the polymorphonuclear leukocytes.

If Diphyllobothrium anemia is true pernicious anemia, a study of it should open up a stimulating discussion of the etiology and pathogenesis of pernicious anemia. And herein lies, perhaps, the greatest significance of further intensive investigation of this type of anemia.

The object of this study is an attempt to present a review of the literature on the subject, as completely as the bibliographic data have been available. It is felt that an endeavor of this kind will relieve future writers of much time-consuming reference hunting and laborious translations, and will facilitate the study of cases. No comprehensive review in the English language has as yet been published.

PART I. BIOLOGY OF DIPHYLLOBOTHRIUM LATUM

The fish tapeworm or broad tapeworm has many synonyms: *Tenia a épine* (Andry, 1700), *Taenia lata* (Linnaeus, 1748), *Taenia membranacea, Taenia tenella* (Pallas, 1781), *Taenia dentata* (Batsch, 1786), *Taenia grisea* (Pallas, 1796), *Bothriocephalus latus* (Bremser, 1819), *Dibothrium latum* (Diesing, 1850), *Bothriocephalus balticus* (Küchenmeister, 1855), *Bothriceophalus cristatus* (Davaine, 1874), *Bothriocephalus latissimus* (Bugn, 1886), *Dibothriocephalus latus* (Lühe, 1899), and *Diphyllobothrium latum* (Linnaeus, 1748). The last term is correct according to modern terminology and will be used hereafter although *Bothriocephalus latus* has been the name most commonly used.

The worm was first distinguished from the Taeniae by F. Glasser in 1603; but the first accurate description, accompanied by artistically finished plates, was given by Bremser in his monograph of 1819. Sommer and Landois worked out its anatomic structure in 1872. Spöring who completed his medical study as early as 1726, made observations about the frequent occurrence in Finland and had the experience that "those people who live on the banks of rivers, rapids and lakes, where there is plenty of fish, suffer from tapeworm more than others." His conception, that human beings got the tapeworm from fish startled the medical body in Sweden to a certain degree, at the time (251). More than one hundred fifty years later, in 1883, Braun (61) demonstrated that *Diphyllobothrium latum* was a parasite in freshwater fish in its plerocercoid stage. But it was only as recently as 1917 and 1918 that the complete life cycle was disclosed by Janicki and Rosen. On this continent the life cycle was worked out by Magath (258, 260) and Essex in 1927.

Anatomy

In the terminal host the head of the worm is usually attached to the intestinal mucosa in the middle portion of the ileum, the remainder of the worm either following the course of the intestinal loops or lying rolled up in clumps.

The average length is 4 to 6 m. but certain specimens may become much longer, up to 20 m. When several worms are present in the same host, each one is usually smaller, often only 1.5 to 2 m. The color is ivory or grayish-white.

The adult worm consists of a chain of flat, rectangular segments, or proglottids, sometimes numbering as many as 3000 to 4000. One end of the chain is narrowed down to the size of a thread and is terminated by a head, or scolex, which has the form of a flattened ovoid, 2 to 4 mm. long and about 1 mm. broad, furnished with two longitudinal grooves, or bothriae, traversing the ventral and dorsal surfaces, but appearing laterally due to torsion of the neck piece. These grooves furnish the means of attachment to the intestinal wall. The segments next to the head are the smallest and youngest and have a rather indistinct structure. In the middle section the mature segments are about 3 mm. long and 10 to 12 mm. broad; they contain a dark brown, rosette-shaped, central, egg-filled uterus, which opens in the mid-ventral line, immediately behind the genital pore. Toward the distal end, the oldest segments grow somewhat narrower and assume a more or less irregular shape.

The uteri of fully developed segments continuously discharge eggs in varying numbers into the intestinal content, with which they usually are well mixed and thus eliminated. In the absence of proglottids in the stools the diagnosis has to be made on the basis of ova through microscopic examination of the feces. When scarce, the ova may be more easily found by some method of concentration (436). They are oval and measure about 65 μ in length and 45 μ in width; they have a brownish color, and are filled with small spherules; the shell is thin, and has at one end a small, hinged lid which may be opened by exerting pressure on the cover glass; at the opposite end a small nodule or localized thickening of the shell may be observed (64, 257).

Life cycle

Ova have been seen to appear in the feces of man on the twenty-fourth day after the infestation (316) and in experimental dogs after three or four weeks (260).

Entering into water the egg hatches and in about two weeks develops into a ciliated embryo, or onchosphere, which slowly swims about. This embryo will die within a few hours unless it is consumed by certain minute crustaceans; *Cyclops strenuous* and *Diaptomus gracilus* (202), *Diaptomus graciloides* (340), *Diaptomus oregoniensis* (125), and perhaps by *Cyclops brevispinosus* and *Cyclops prasinus* (125). These are first intermediary hosts in the life cycle of the parasite, and within their body cavity the embryo develops into the procercoid stage in about three weeks (68).

In turn the crustaceans which harbor the young parasites are eaten by fish which serve as second intermediary hosts, the larvae developing into the stage known as the plerocercoid. Infestation has been found to occur in *Coregonus albula*, *Coregonus lavaretus*, *Lota vulgaris*, *Lota maculosa*, *Salmo salar*, *Salmo umbula*, *Salmo vitreum*, *Perca fluviatilis*, *Trutta vulgaris*, *Trutta lacustris*, *Esox lucius*, *Stizostedion vitreum*, *Thymallus vulgaris*, *Onchorhynchus perryi* (Japan) and others (64, 68, 206, 258, 260, 444). From the intestinal tract of the fish these larvae penetrate into the muscle mass where they come to rest. The fish with the larvae are then consumed by fish-eating human beings, dogs, cats, bears and foxes, and perhaps by other carnivora (64, 68, 447, 459, 463).

Successful experimental infestation has been undertaken in human beings (232, 288*a*, 317), in dogs (62, 125, 258, 259, 260), in cats (62, 288*a*,

447) and in bears (447). It may be mentioned in passing that Hobmaier by experiments has proved that predaceous fish in later life also may become infested through ingestion of infested fish.

Plerocercoids have been found not only in the meat of the fish but also in the egg leaders, free on the mucosa or mixed with the eggs. Thereby another mode of infestation is made possible (180) and eating of caviar is given as one source (64, 459).

Mode of infestation

There is no danger of infestation if the fish is properly heated or frozen. The plerocercoids are killed at a temperature of +51° to 53°C. (206). (Schor gives +54° to 55°C.) and also die when exposed to a temperature of −9°C. for forty-eight hours. However, they can readily stand temperatures between +50°C. and −3°C. (Schor stated that the plerocercoids are killed at −3° to +1°C. when exposed at least two days, whereas ten minutes of frying or cooking is sufficient to kill the larvae). Placed in a 10 per cent solution of sodium chloride the larvae seem to thrive; after forty-eight hours of exposure, even if they are found in a stiff and shrunken condition, they can be revived; in a 15 per cent solution they are still alive after five hours, and in a 20 per cent solution after one and a half to two and a half hours. They are, on the other hand, found dead in well dried and smoked fish, according to Kajava, whereas others have obtained living plerocercoids from smoked fish (64, 459). Thus the infestation most likely is caused by the consumption of insufficiently sun-dried (64), mildly salted (206), pickled (163), smoked fish or fish not sufficiently frozen. Caviar is a common source of infestation in Roumania (234). The frequent infestation in Jewish housewives in contradistinction to the rarity of infestation in Jewish males is explained by the fact that "gefüllte Fisch" is a favorite dish among Jews and sampling of the raw fish in the preparation of it is extensively practiced, whereas plerocercoids are not alive when the fish finally is served (19). Salted, smoked and cured fish in Finland is everyday food for the middle classes, and delicatessen for the upper classes, thus explaining the tremendous prevalence of the infestation in that country (163). The dietary habits in the other Baltic States are similar.

Duration of life

The lifetime of *Diphyllobothrium latum* as an intestinal parasite is difficult to determine, and has been variously estimated, as it is practically impossible to rule out reinfestation. Commonly patients in middle and advanced age state that they have had tapeworm since childhood or "as long as they can remember" (11, 346, 354, 380). But dietary habits which make infestation possible pave the way for reinfestation as well.

Geographic distribution

The parasite has been reported endemic in various parts of all continents except Australasia. Two important centers of distribution have been determined: the Baltic shores, consisting of northern and middle Sweden (250), Finland, Russia, Esthonia, Latvia, Lithuania, Eastern Prussia, and French Switzerland (64). From the former the distribution has radiated into Russia, Russian Turkestan (138), Poland (299), Denmark (218), and Iceland. The Netherlands (81), Belgium (39, 142), Ireland (148) and the United States, Canada and Alaska (348, 443–449, 459, 463, 464); from the latter into France near the Swiss border (335), Southern Bavaria (442), Austria-Hungary (336) and northern Italy (64). Japan is heavily infested (193, 266, 293). *Diphyllobothrium latum* has also been encountered in Siberia and Manchuria (133), Palestine (220), British Bechuanaland around Lake N'Gami (64, 68), Angola (468), Uganda (107), Madagascar (21), Philippine Islands (401), and the West Indies (60). Herff has expressed the belief that it is indigenous in Mexico.[2] In Brazil (Bahia) infestation has been observed in a sailor from the Baltic region (412). One case of so-called *Dibothriocephalus parvus* in a young male Syrian was observed at the General Hospital in Launceston, Australia, in 1898. This man, however, had recently arrived in that country so it is questionable whether he got his parasite there (122). *Diphyllobothrium parvum* is, according to Magath (260), identical with *Diphyllobothrium latum*, the former representing the latter before the terminal segment has sloughed. Kurimoto in Japan and Leon (235) in

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[2] Alvarez informed me that it is not uncommon in Syrians in the State of Sonora, Mexico.

Roumania have observed Diphyllobothria with double genital apparatus in man, and Leon (234) also noted other abnormalities in the structure of the worm.

Finland occupies a peculiar position among the countries mentioned, as it is the one most heavily infested. That the fish tapeworm has a foothold of long standing in that country is evidenced by Spöring's observations in the first half of the eighteenth century. And according to the more recent reports a great proportion of the population is infested. There are certain districts with infestation of practically 100 per cent; in a large portion of the country the frequency of infestation is about 50 per cent, whereas the average for the whole country is 20 to 25 per cent (120, 163, 409). This means that there must be 500,000 to 750,000 carriers of parasites in Finland alone. Certain sections of the other Baltic countries are almost as heavily infested. Sander found 25 per cent infestation in St. Petersburg. In Turkestan and Japan the broad tapeworm is the most common of intestinal parasites (64). The frequency and distribution of this tapeworm have been on the decrease in certain areas of the old world, such as Paris and Geneva (64); in the new world with mass importation of carriers of parasites and the persistence of dietary peculiarities favorable for the maintenance and further dissemination of the infestation, a steady increase is being observed (460, 461).

PART II. PATHOGENICITY OF DIPHYLLOBOTHRIUM LATUM

Nonanemic carriers

Children are generally considered free of infestation, but Ehrström (118) has stated that although increasing frequency with age is recognized, no age is exempt, not even infancy.

The majority of carriers enjoy perfect general health. In a certain number, however, are more or less disturbing phenomena, which may be grouped under different headings: manifestations from the nervous system, the digestive system, or the blood system (64, 68, 438).

Nervous and mental manifestations. These seem to occur in persons with a predisposition for such disorders, and children are believed to be more susceptible than adults. The symptoms are greatly varying and usually functional in type. Consciousness of the presence of the parasite may probably suffice for the development of neurotic states in carriers if such parasitism represents an unusual condition in a particular locality. It is known, however, that also in countries in which such unfamiliarity does not exist, where the worm is taken more or less for granted, functional disorders may occur and may range in degree from the mildest to the most severe forms. Analogous manifestations are seen in dogs, in whom intestinal parasitism often causes fits. Tonnel saw immediate improvement of the mental condition of a maniac patient with family tendency to mental disorders, following administration of a vermifuge. Brumpt (68) enumerated, among others, the following conditions as resulting from the parasitism: epileptiform crises, hysteriform crises, choreatic phenomena, convulsive states accompanied by headache and constipation (meningisme vermineux), catalepsy, various types of paralysis, strabismus, aphonia, dyspneic attacks, visual disturbances (illusions, diplopia, xanthopsy, amaurosis), pruritus of nose and anus, and urticaria. The proof that all these conditions may be occasioned by the tapeworm is seen in the fact that they improve and disappear following elimination of the parasite.

Manifestations in the digestive tract. These vary markedly, not only in different persons, but from time to time in the same person. In Finland the dietary regimen helps the parasite in producing gastro-intestinal symptoms; persons there consume, as a general rule, too much sour milk, too sour bread, raw and too salty fish, and too much coffee (163). The popular belief is that bulemia regularly accompanies infestation with tapeworms; more frequently, however, either total or partial lack of appetite for certain foods is noted; salivation is sometimes increased. Ructus, pyrosis and nausea, epigastric distress and pain with or without apparent relation to ingestion of food are observed; likewise sensation of pressure or of strange movements, colic and crisis-like attacks, especially in the umbilical region are observed. Abdominal pains, with associated tenderness (25, 26, 173), may simulate gastric ulcer (309), appendicitis (169), or ileus (144). Vomiting may occur after meals, or on an empty stomach in the morning, and may be so strained and violent as to cause part of the parasite to be ejected with the emesis (184). The movements of the bowels are inclined to be periodically irregular; constipation or diarrhea may prevail or they may alternate (25, 381). Becker (25) remarked that in his observation of a series of ninety-seven carriers only twenty-one had no particular complaints, whereas forty-three gave histories of periodic diarrhea or diarrhea alternating with constipation. Eighteen had almost continuous diarrhea and fifteen almost continuous constipation.

Symptoms from disorders of the mouth and tongue are not uncommon in carriers without anemia and deserve special mention. Cederberg (86, 86a) gave a frequency of about 10 per cent and Becker (25, 26) of more than 16 per cent. There seems to be an increase in frequency with age. In Finland infestation with tapeworm is made responsible for the majority of instances of stomatitis-glossitis observed. Even laymen are beginning to realize the etiology of the condition and to subject themselves to treatment directed against the cause which in practically all instances suffices to relieve them. Kamala is becoming regularly used in the treatment. Symptomatic and palliative treatment is remarkably ineffective. Stomatitis and glossitis are characteristic features of infestation with Diphyllobothrium and often the only symptom manifested. The onset is usually insidious but may, at times, be rather sudden; the attacks vary greatly in severity, both from time to time in the same person and in different persons; the course has a tendency to be intermittent. Mild cases of subjective complaints, more than objective pathologic changes, Cederberg designated as formes frustes. Cases are also observed in which the marked pathologic changes are not associated with correspondingly severe subjective complaints. The symptoms, in severe cases, are usually hypersensitivity of the mucosa, with burning and stinging pain, increased salivation, and swelling and tenderness of the submaxillary lymph nodes. The oral mucosa is edematous, hyperemic, sometimes superficially ulcerated, the gums receding from the teeth and easily bleeding. The tongue is swollen and fiery red; deep grooves traverse the surface, and the edge is indented from the pressure of the teeth. On account of the oral condition interfering with the intake of food the patient may be losing weight and strength and may become pale. According to the situation of the pathologic involvement, Cederberg classified the cases of oral disorders under the headings of (1) glossitic, (2) gingival, (3) buccal, and (4) generalized. The gingival form has a decidedly bad effect on the teeth. The buccal form occasionally is associated with perioral dermatitis. The average case, fortunately, is not fulminant and severe. Usually rubor is observed in spotted distribution, at the tip or along the edge of the tongue, or on the inner surface of the cheeks and lips. Blisters and aphthous eruptions may be seen. The tongue often becomes smooth and glossy, evidently due to atrophy of the papillae. The grooves and furrows traversing the surface have given it the name of lingua geographica; the red coloring has given it the term of beefsteak tongue.

Schauman and Saltzman (389) stated that in many instances the tongue will resume its original, normal appearance, to a certain degree, following expulsion of the worm.

The problem of achlorhydria in Diphyllobothrium anemia has stirred several investigators to studies of the gastric secretion in nonanemic carriers. Of seventeen cases Törnell found achlorhydria in twelve (72.6 per cent), and only traces of hydrochloric acid in the remaining five cases. This corresponds closely to the observations of Latzel and Staniek in fifty cases of nonanemic persons infested with *Taenia saginata*: 36 per cent had achlorhydria; 54 per cent, hypochlorhydria; 8 per cent, normal acidity, and 2 per cent, hyperacidity. In four cases in which acidity was low this returned to normal after expulsion of the parasite. Tallqvist (428) obtained entirely different results. Of thirty-eight cases of Diphyllobothrium carriers he found, respectively, hypoacidity and anacidity in 13.2 per cent, normal acidity in 68.4 per cent and hyperacidity in 18.4 per cent. J. Grönberg (163) examined the gastric acidity in 389 dyspeptic patients, excluding those with carcinoma of the stomach and pernicious anemia. Of these patients, fifty-four had simultaneously *Diphyllobothrium latum*. He compared the two groups of patients, the one with, the other without, the worm, and discovered that the helminthiasis had no appreciable influence on the gastric secretion.

In twenty-two cases of dyspepsia associated with *Diphyllobothrium latum* J. Grönberg determined, in addition, the gastric acidity both before and from seven to fifty-two days after successful elimination of the worm. In half of the cases there was no appreciable difference between the two determinations. In five cases the acidity was slightly higher before than after elimination of the parasite, and in six cases the opposite took place. But, in all, the changes were too slight as to be of any significance. Eight years earlier Schauman and A. Grönberg had arrived at a similar conclusion.

Manifestations in the blood. Cederberg (86) and Becker (25, 26) stated that many of the carriers, even though they may not be definitely anemic, are somewhat pale, sometimes with a tinge of yellow. Warthin (464) remarked that all carriers to whom his attention was called appeared more or less anemic. It seemed important, therefore, to investigate the blood in order to determine eventual hematologic changes. The blood picture in tapeworm carriers has been carefully studied by Ragosa in twenty-six cases and by Becker (24, 25) in 213 cases. Both used standardized methods and made comparisons with their own normal controls.

The number of erythrocytes usually is found to be somewhat decreased, but may also be normal. The concentration of hemoglobin as a rule is somewhat diminished, but cases are also seen in which it is normal or even greater than normal. In the majority of cases the color index is slightly heightened, but it may remain normal. When the number of erythrocytes is nearly normal, they are not definitely changed in appearance. With increasing deviation from the normal count there is some evidence of poikilocytosis, anisocytosis, macrocytosis (confirmed by Passey and Braine, Hampson and Shackle but not by Emmons) and polychromatophilia. Normoblasts may occur; they have been observed by Ragosa in six cases, and by Becker (24, 25) in two cases. In this study a dividing line between nonanemic and anemic carriers has been drawn arbitrarily at an erythrocyte level of 4,000,000 for each cubic millimeter of blood.

The leukocytes are generally slightly decreased in absolute numbers, although there are many exceptions. Whereas Becker (24, 25) did not find alteration in the differential count, Ragosa saw a distinct tendency toward relative lymphocytosis. This was present to a marked degree in the case of Bendix.

The behavior of the eosinophils is as inconstant as it is interesting. A great many writers seem to have expected eosinophilia. Ebbel pointed out emphatically that eosinophilia is not associated with infestation by Diphyllobothrium. Ragosa found a normal eosinophil count in 70 per cent of his cases, a count below 1 per cent in 15 per cent, and a count between 5 and 7 per cent in the remaining 15 per cent. Becker (24, 25) noted a moderate tendency toward eosinophilia, and that it was more common among persons who had not reached the age of fifteen years than among older persons. Leiper in experimental human infestation found initial eosinophilia, in which the proportion of eosinophils rose to 15 or 16 per cent in three weeks, and thereafter decreased to about 2 per cent. At this level, the proportion of eosinophils remained for the balance of the duration of the infestation (as long as five and a half years).

Between a fully normal blood picture and one characteristic of well defined pernicious anemia, all intermediary steps have been observed, and at no point is it possible to determine where the former ends and the latter begins. Ragosa therefore significantly called the condition of the blood in nonanemic carriers a forme fruste of the typical blood picture in advanced Diphyllobothrium anemia. Becker (24, 25), did not find as high an incidence of changes in the blood as Ragosa found.

In only a minor proportion of carriers, no changes at all are seen; the changes increase, on the whole, progressively with the lowering of the number of erythrocytes (334). An altered blood picture usually returns to normal after the worm has been expelled (24, 25).

Ragosa also examined the blood in thirty-nine cases of infestation with taenia and discovered similar changes but in a smaller percentage and to a less degree.

Kovarski recorded a case in which there was rather pronounced hemorrhagic diathesis, expressed in hemorrhages from the kidneys and from the throat, which he attributed to the presence of the fish tapeworm in the intestine. Proof of the causal relationship was not offered, however.

Chronic myelogenous leukemia has been observed by Stukowski in a case in which the stools contained segments of Diphyllobothrium and a vermifuge caused one fish tapeworm and one *Taenia saginata* to be recovered. In this solitary case of leukemia and parasitism the association of the conditions was probably purely coincidental.

The serology in tapeworm carriers has been studied extensively. Many of the clinical manifestations for which tapeworms have been made responsible are of such a nature as to conform with the hypothesis that the worms somehow exert a toxic effect on the organism which harbors them. They are generally believed to secrete, or by disintegration to yield, one or more toxins, which on continuous resorption into the blood stream may attack the various systems and give rise to such symptoms as those contained in the summary that has been given. That toxic resorption takes place in carriers in whom anemia develops is a common contention, but Ragosa, Becker (25), and Cederberg (86) feel that such resorption occurs in nonanemic carriers as well. It is known that the blood serum possesses some detoxicating properties, and that the organism owes its resistance to toxins to these protective mechanisms. One way of approaching the problem of the toxicity of the tapeworms, therefore, would be to try to determine the properties of the serum of the carriers in this respect.

Isaac and von den Velden, in 1904, observed a patient with mild Diphyllobothrium anemia in whom erythrocytes numbered 3,200,000 in each cubic millimeter of blood and the concentration of hemoglobin was 65 per cent. They prepared a solution of the worm by means of antiseptic autolysis, and mixed it with blood serum from the patient. This serum gave a positive precipitin reaction in a dilution of 1:1000. A rabbit was given repeated injections with Diphyllobothrium solution intravenously. In all, this animal received 20 cc. of solution, and serum from this rabbit also gave a positive precipitin reaction. Controls were negative. This work was commented on in an editorial (110) and was considered a distinct advance in the study of infestation with Diphyllobothrium.

The same year and following years Langer and Fleckseder and Stejskal studied the precipitin reaction in carriers of taeniae. Fleckseder and von Stejskal worked with extract of *Taenia saginata* and serum of rabbits that had been given injections of this extract, and obtained, experimentally, a positive precipitin reaction, similar to that obtained by Isaac and von den Velden. Langer, on the contrary, got negative results in twelve cases, six with *Taenia solium* and six with *Taenia saginata*, and similar tests in dogs and cats which harbored a great number of taeniae also came out negatively. Langer's conclusion, therefore, was that specific formation of antibodies did not take place in the types of helminthiasis investigated by him.

K. Meyer (279), in 1913, restudied the precipitin reaction in immune serums made against *Taenia saginata*, using tapeworm lecithins in high dilutions, and obtained precipitation. He also found that heterologous serums cause precipitation, but only in strong solutions, and that heterologous lecithin is not at all precipitated by immune serums made against tapeworm. In further experiments on rabbits he found that this formation of antibody was little and inconstant when the animals were immunized with the lecithin group, whereas with the kephalin group it reached equally high values as in protein immunization.

Tallqvist (432, 433) could not demonstrate antibodies in animals that had been given injections of Diphyllobothrium lipoid, but found that protein from the same parasite had definite antigenic properties.

Sachs-Georgi's reaction was tested by Becker (28) in 1922. This proved not to be a specific immunization reaction, for serums from patients with diabetes, pernicious anemia, or diffuse nephritis reacted similarly. His experiments showed that one could obtain, with suitable alcoholic extracts of the fish tapeworm, such a reaction as usually not to produce precipitation, in serums from Diphyllobothrium carriers, whereas in serums from persons who were not carriers certain precipitation usually would occur. Becker's explanation of this phenomenon, if I have interpreted him rightly, is that the precipitation in Diphyllobothrium carriers is inhibited by a substance present in the serum which acts as a protective colloid. But according to general usage of the terms "negative" and "positive" in this test Becker usually got negative reaction in Diphyllobothrium carriers and patients with diabetes, pernicious anemia and diffuse nephritis, whereas others, who were not carriers, gave a positive reaction. It seems to me that, at the most, one may conclude from Becker's results that Sachs-Georgi's reaction does not give any indication of whether or not resorption of worm substances occurs in carriers.

Meyer (275–278, 280) extended his researches to comprise complement fixation tests in tapeworm (*Taenia saginata*) carriers, and with alcoholic extracts of worms he obtained a positive reaction in a third of his cases. Serums from these subjects gave the same positive reaction with fluid from echinococcus cysts and with watery and alcoholic extracts of the wall of echinococcus cysts. Furthermore, he found that serum from patients infested with echinococcus reacted negatively with extract of tapeworm as well as with extracts of echinococcus cysts at first, but it reacted positively after an operation whereby cysts were opened and fluid was emptied out into the peritoneal cavity.

Controls with normal serums did not give positive reactions. The same complement-fixing antibodies could be obtained experimentally by immunization of rabbits with alcoholic extract of worms. The tapeworm antigen, he concluded, was a lipoid of the lecithin group. The reaction was specific, not to species but to genera.

Ghedini and others confirmed Meyer's conclusions, but Paccanaro got positive reaction even in subjects who were not carriers. Weinberg and Parvu found specific antibodies in the serum of 50 per cent of the animals examined which harbored parasites. Busson obtained negative reaction in carriers who had harbored their parasites over a long time, or in large numbers, without any evidence of inhibited resorption of an eventual antigen. Only in a certain small percentage of carriers did he see a positive reaction. Busson also stated that normal rabbit serum frequently had the property of complement fixation with alcoholic extracts of tapeworm or cardiac muscle, and this normal property could be intensified by injections of indifferent substances like leucine, tyrosine or extract of heart muscle. Serum of persons with syphilis was found to give complement fixation with alcoholic extracts of tapeworms, echinococci and cardiac muscle.

Barit repeated Meyer's experiments, with negative results. He was unable to find an explanation of the divergence between his and Meyer's results. Most of his subjects had harbored Diphyllobothrium for years; one expelled seventeen specimens after conclusion of the experiments. The objection that perhaps not sufficient resorption had taken place could not be upheld, for in some of the cases severe anemia had developed. And yet, not once in the eleven serums from carriers, tested against several extracts of tapeworm, did he see indisputable specific complement fixation.

Ignatius obtained the same negative result. Jerlov tried out the complement fixation test on eleven Diphyllobothrium carriers, on ten taenia carriers and on four patients with parasitism of unknown type. For antigen, he used either an extract, in alcohol and ether, of dried worm powder or an ethereal extract of the moist worm. Both extracts were evaporated to dryness and were redissolved in sufficient physiologic solution of sodium chloride to make a concentration of 10 per cent. He found that Diphyllobothrium carriers often gave a positive reaction, although sometimes the result was negative. In those who were not carriers, the reaction never was strong. Taenia carriers often gave negative reactions. Positive serum often reacted more strongly with specific antigen. Occasionally, however, the serum of a Diphyllobothrium carrier reacted more strongly with taenia antigen than with Diphyllobothrium antigen, or the serum of a taenia carrier, more strongly with Diphyllobothrium antigen than with taenia antigen. The Wassermann reaction was negative in serum from carriers studied. Alcoholic extract of Diphyllobothrium substance gave positive reaction with serums which were Wassermann-positive. Jerlov concluded that a serologic diagnosis of helminthiasis (Diphyllobothrium and taeniae) might be made in some cases which were Wassermann-negative; a positive result would be considered conclusive and a negative result, not conclusive.

Becker (26), in 1920, repeated Meyer's and Jerlov's experiments in a number of cases and got "almost complete hemolysis" in two Diphyllobothrium carriers tested against Meyer's antigen, and a faintly positive reaction with one of Jerlov's extracts. Tests in all the other cases came out negatively. He also tested the antigenic properties of Seyderhelm's bothriocephalin but with negative result. Concluding that the antigenic properties must be bound to proteins, he turned to saline extracts and hydraulically expressed juice of the worm substance, prepared in different ways. The material examined came from fifty-eight carriers and ninety-two control subjects who were not carriers. In the former group, 53.45 per cent gave positive reactions, whereas in the latter, only 10.87 per cent gave positive reactions. When the carriers were subdivided into two groups, one comprising subjects with uncomplicated helminthiasis and one comprising subjects with helminthiasis and some complication, an interesting fact was disclosed. The complement fixation test was found to be positive in 68.42 per cent of the former subgroup and in 45 per cent of the latter. Thus, it looks as though a healthy carrier may produce more antigen than one whose resistance is lowered by some kind of complication. In regard to the 10 per cent of positive reactions in subjects who were not carriers, Becker mentioned the possibility of a person harboring the parasite in spite of negative examination of the stool, that a person may have had a worm previously and might retain antibodies in the blood, or that he might have nonspecific antibodies in his serum.

Becker considered the reaction nonspecific, since the serum from taenia carriers also could give positive reactions with Diphyllobothrium antigen. Le Bas could not demonstrate any antibody formation in the serum in two cases of experimental human infestation.

After reviewing the literature on the serology in tapeworm infestation, it becomes apparent that as yet no method has succeeded in definitely determining the presence of antibodies against worm substances in the blood serum of the carriers.

In regard to the blood chemistry in tapeworm carriers, Becker (24), in 1914, discovered urobilinogen and urobilin in the urine of some Diphyllobothrium carriers. By him, this was interpreted as a possible indication of an abnormally high destruction of erythrocytes in the absence of anemia. Pursuing this thought, he determined the potassium content of the blood serum in carriers (30) in order to see whether the content of potassium was increased. An increase would speak strongly for increase in destruction of erythrocytes, which have a potassium-content twenty times greater than that of the serum. The results of this research were not at all uniform and a definite conclusion could not be drawn, although in five of thirty-one carriers there was an increase in potassium ranging from 4.5 to 50 per cent. Becker previously had found an increased potassium-content of the blood serum in cases of kryptogenetic pernicious anemia. In one case, quantitative analysis of the blood serum, two weeks after successful anthelmintic treatment, gave a normal amount of potassium, whereas previously the content of potassium had been high.

ANEMIC CARRIERS (DIPHYLLOBOTHRIUM ANEMIA; BOTHRIOCEPHALUS ANEMIA)

By far the most interesting and conspicuous complication in infestation with *Diphyllobothrium latum* is anemia the details of which presently will be described. Most of the information is derived from Schauman's two monographs (382, 389) which are understood to be the main source, additional references being specifically mentioned.

Geographic distribution. One would naturally expect the highest frequency of anemia in the most heavily infested areas. This is generally the case, inasmuch as Finland and the southeastern Baltic states contribute the greatest number of cases. But a compilation of the recorded cases, arranged according to the national origin, shows a strange and remarkable feature (table 4).

Japan, Italy, Roumania, and Sweden, all heavily infested, at least in parts, have little to offer in regard to anemic complications. In an article published in 1887, Murata called attention to the great prevalence of infestation of human beings with *Diphyllobothrium latum* in Japan and added that he had never seen nor heard of anemia resulting from this parasitism in his country. Von Noorden five years later in a consideration of the relationship between anemia and fish tapeworm, called attention to the peculiar fact that Japan, in spite of the high frequency of the parasite, had not a single case of anemia due to this worm on record. Schauman and Saltzman (389) made the statement in 1925 that Kusimoto had expressed himself to the effect that Diphyllobothrium anemia was not very rare in Japan. Nevertheless, I have been able to find only one report of such a case, that written by Mat-

TABLE 4

Nationality of all patients reported with Diphyllobothrium anemia

COUNTRY	NATIVE COUNTRY	IMMIGRANTS TO AMERICA	TOTAL	COUNTRIES WITH HEAVY INFESTATION
Finland	370+	21	391+	Yes
Russia, Poland and Baltic states	71+	7	78+	Yes
Germany	42	1	43	Yes (eastern Prussia)
Switzerland	12		12	No
Sweden	5	1	6	Yes (northern half)
Roumania	2		2	Yes
France	1		1	No
Austria		1	1	No
Norway ?	1		1	No
Denmark	1		1	No
Italy	1*		1	Yes (certain districts)
Japan	2 or more		2 or more	Yes
?	†	5	5	

* Reported in Switzerland.

† Nationality in Minot's and Kerr and Falconer's cases not given.

sumoto. In his report he mentioned that an additional case of similar anemia, and with fatal outcome, had been observed in the same locality as that in which his case occurred. Kurimoto recorded the case of a severely anemic patient who harbored *Diplogonoporus grandis* (a freak variety of *Diphyllobothrium latum*, having a double set of genital pores) in his intestine. Bard reported Diphyllobothrium anemia in an Italian-born young man who had spent years in Switzerland before he was taken ill. Babes reported the only two Roumanian cases recorded. Sweden which in its middle and northern portion has an infestation of approximately the same degree as that of Finland has only six native cases on record.

How is this strange incongruity to be explained? Is there a difference in toxicity of the worm, in individual resistance to the supposed toxin, in racial stamina and predisposition, or in climatic or hygienic conditions?

In the figures for Finland and Russia there are some inaccuracies. Schauman and Salzman had, up to 1925, compiled 326 cases, all of which were apparently in native Finlanders. Among these cases are evidently all the cases previously reported by Finnish investigators under Schauman's direction and probably the cases reported by Runeberg (363, 366) prior to Schauman's time. Therefore, when I add to this number Orlowsky's case and the cases recently reported by Becker (29), Becker and Tötterman, Lindström (243), Seppä (402), and Saltzman (374) forty-four in all, besides the American reports of anemia in Finnish immigrants, twenty-one in all, I am at least sure not to have exaggerated the total figure. Unfortunately not all of the Russian reports of cases have been available to me, which makes the figure I give for these cases certainly too low. For the sake of completeness, however, I have included references to these cases in the bibliographic list. The nationality of Minot's (285) and Kerr and Falconer's patients was not given.

American and Canadian cases of Diphyllobothrium anemia are summarized in table 3. European cases have been recorded by: Botkin (55, 56), Reyher, Mitzkuner, Runeberg (363–366), Lichtheim, Roux (359), Schapiro, Podwissotzky, Kissel, Shimanskiy, Mueller, Ketcher, Westphalen (470, 471), Dehio (99, 100, 101), Ravitsch-Shtsherbo, Winiarski, Minnich, Pariser, Wiltschur, Eckert, Biruli, Vlayeff, Natanson, Schauman (381), Askanazy, Babes, Faber (130, 131), Shiperovich, Sievers and von Bonsdorff, Ewald (127), Bruhn-Fåhraeus, Neubecker, Aall, Warfvinge, Jawein, Sawjalow, Bard, Zinn, Chemolossoff, Rosenqvist (354), Courmont, Courmont and André, Orlowsky, Isaac and von den Velden, Fischer, Galli-Valerio, Holsti (185), Tonnel, Sahli (369), Liubarskiy, Ragosa, Hülse, Appelberg (8, 9), Herzog, Cramer, Montandon, Grünmandel, A. Grönberg (162), Rosenow (351), Schauman and Saltzman, Becker (29), Becker and Tötterman, Seppä, Mikulowski, Lind (240), L. J. Lindström (243), Belonogowa, Schottmüller, Becker (22) and Saltzman (373, 374). The total number of cases on record has now reached approximately 550.

Cryptogenetic pernicious anemia seems to have a racial preference; it occurs more commonly in northern Europe and North America than elsewhere (94, 389). Statistical information regarding incidence of pernicious anemia in most countries other than the above mentioned regions is largely lacking. The disease is said to be very uncommon in Brazil (215), in China (88), and among negroes (484); Jews are supposed to be very resistant to it.

In the years 1895 to 1912 twenty-nine cases of cryptogenetic pernicious anemia were observed at the Medical Clinic of Makarow, at Jena University, whereas in the years 1883 to 1908 the Medical Clinic of Schauman at Helsingfors, which is smaller than the former, observed ninety-three cases of cryptogenetic pernicious anemia and in addition 221 cases of Diphyllobothrium anemia. Table 5 illustrates the greater frequency of pernicious anemia (cryptogenetic and Diphyllobothrium types) in Finland as compared with the number of hospitalized patients.

Sex. There seems to be no preference for either sex in the occurrence of Diphyllobothrium anemia. Of 471 cases, in which the sex of the patient was given, 245 (51.9 per cent) were in males and 226 (48.1 per cent) in females. In Schauman's (380) series of 1894 the distribution was 63.89 per cent and 36.11 per cent, respectively, and in Schauman and Saltzman's series of 1925, including Schauman's old material, it was as follows: total 326; males 160 (49.10 per cent), and females 166 (50.90 per cent).

The distribution as to sex of cases of cryptogenetic pernicious anemia is not so clear cut. English and American writers are inclined to consider males more susceptible to this disease. Gulland and Goodall gave the frequency as 65.8 per cent in males, and Cabot (76), as 62.5 per cent in males. The German and Finnish statistics give a corresponding preponderance to females; that is, Lazarus gives 62.5 per cent as the proportion of cases in females, Schauman and Saltzman 64.6 per cent, and Lindström (242) 62.47 per cent.

The preponderance of one sex over the other is about the same in each set of figures and each neutralizes the other when the two are added together. Therefore, it appears impossible to conclude, from the data given, that sex preference is in existence even in cryptogenetic pernicious anemia.

TABLE 5

Frequency of pernicious anemia in patients in hospital in different countries (Schauman and Saltzman)

SOURCE OF MATERIAL	MATERIAL	PATIENTS WITH PERNICIOUS ANEMIA					
		Cryptogenetic		Diphyllobothrium		Both types combined	
		Number	Per cent	Number	Per cent	Number	Per cent
Berlin	148,000*	274	0.19			274	0.19
Sweden	102,756*	496	0.48			496	0.48
Finland	44,087*	194	0.44	326	0.74	520	1.18
Jena (Makarow)	1895–1912†	29				29	
Helsingfors (Schauman)	1883–1908†	93		221		314	

* Consecutive cases.

† Years during which cases were collected.

Age. Diphyllobothrium anemia is very rare in childhood; only three cases have been recorded in patients who were in the first decade of life. The greatest number is found in the third decade of life (table 6). In order to obtain a more correct impression of the true age distribution, however, one should consider the percentage distribution, of the disease and of the population, and calculate the proportion of the former as compared with the latter. This will give indexes more truly comparable than absolute numbers and percentages. Graphic curves of these indexes, shown in figure 1, have essentially two peaks: the first one they have in common, in the third decade; the other appears either in the sixth decade, as in Lindström's (242) curve or in the fifth decade, as in Schauman and Saltzman's and in mine. From the second peak all curves decline, Lindström's the least. Compared to these observations, the curve for cryptogenetic pernicious anemia assumes a different contour. This type of pernicious anemia is much more definitely a disease of age. The curve rises steadily to its peak in the seventh decade, as in Lindström's, or in the sixth decade, as in Schauman and Saltzman's, and drops off decisively beyond the point which represents seventy years, probably because most of those who were destined to die from pernicious anemia had died in an earlier decade.

TABLE 6

Age distribution of cryptogenetic pernicious anemia and Diphyllobothrium anemia in Finland (Schauman and Saltzman)

AGE GROUP	PERCENTAGE DISTRIBUTION OF POPULATION IN 1910	CRYPTOGENETIC PERNICIOUS ANEMIA			DIPHYLLOBOTHRIUM ANEMIA			BOTH TYPES COMBINED		
		Cases	Per cent	Index*	Cases	Per cent	Index*	Cases	Per cent	Index*
years										
0– 9	23.97	1	0.52	0.022	1	0.31	0.013	2	0.39	0.016
10–19	19.32	5	2.58	0.13	45	13.80	0.71	50	9.62	0.50
20–29	16.66	32	16.49	0.99	85	26.07	1.56	117	22.50	1.35
30–39	13.56	40	20.62	1.52	60	18.41	1.36	100	19.23	1.42
40–49	9.76	41	21.13	2.16	64	19.63	2.01	105	20.19	2.07
50–59	7.93	42	21.65	2.73	46	14.11	1.78	88	16.92	2.13
60–69	5.59	28	14.43	2.58	16	4.91	0.88	44	8.46	1.51
70 and more	3.21	5	2.58	0.80	9	2.76	0.86	14	2.69	0.84
Total........	100.00	194	100.00		326	100.00		520	100.00	

* Proportional index relative to population.

The reason for the first peak in the curve of Diphyllobothrium anemia, and its absence in that of cryptogenetic pernicious anemia is not obvious. It is possible that the cryptogenetic etiologic factor, in some way, is associated with the factor of time, whereas the parasite may exert its evil influence at any period of a person's life.

Occupation and hygienic conditions. Schauman and Saltzman's statistics show that Diphyllobothrium anemia, like cryptogenetic pernicious anemia, occurs in persons from every social stratum. No particular type of work seems to predispose to this disease. Poor and rich, physical and mental workers, city dwellers and country folk, laborers within doors and out of doors all have their share. There seems to be no relative preference. Poor hygienic conditions were emphasized by Cramer in his report of three sisters who had been living together and in whom Diphyllobothrium anemia developed simultaneously.

This point is brought out in the consideration of the etiology of cryptogenetic pernicious anemia as well. Work in lead and about gas houses (168), and carbon monoxide poisoning (160) are referred to as factors of importance.

Inadequate food, quantitatively and qualitatively, was mentioned by Biermer (46) as a possible causal factor in the etiology of cryptogenetic pernicious anemia, and this viewpoint has of late received strong support by those who have seen good results from treatment with a diet high in calories and vitamines. Such deficiency of food was specifically pointed out as probable in tapeworm anemia by Cramer, and recently also by Richter, Maurer and Eyl.

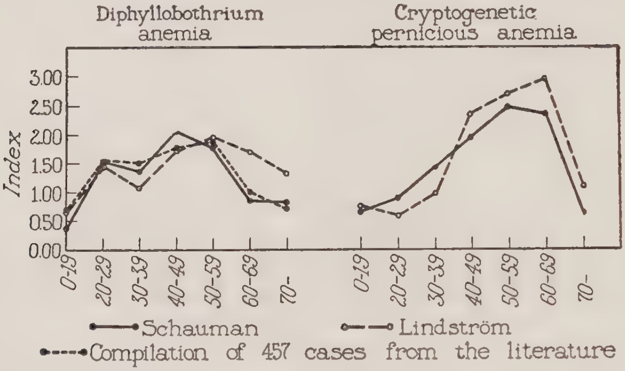

FIG. 1. RELATIVE PROPORTIONAL INDEX OF AGE DISTRIBUTION

Seasonal influences. There is a definite tendency to greater frequency of Diphyllobothrium anemia in spring and summer. Figure 2 illustrates this clearly. The same tendency is shared by the cryptogenetic type of pernicious anemia. An explanation of this phenomenon is not easy to give. The spring fatigue which is commonly experienced in northern countries may have a bearing on it. The long and dark winters in a northern climate may play a part.

Morbidity. Lindström (242) has plotted curves (fig. 3) which show the incidence of Diphyllobothrium anemia and of cryptogenetic pernicious anemia in four hospitals in Helsingfors. These curves demonstrate that the incidence of Diphyllobothrium anemia was steadily declining between 1898 and 1927, whereas the occurrence of cryptogenetic pernicious anemia was approximately the same from year to year until 1910, after which there was a marked increase. He stated that his curve for cryptogenetic pernicious anemia corresponded closely

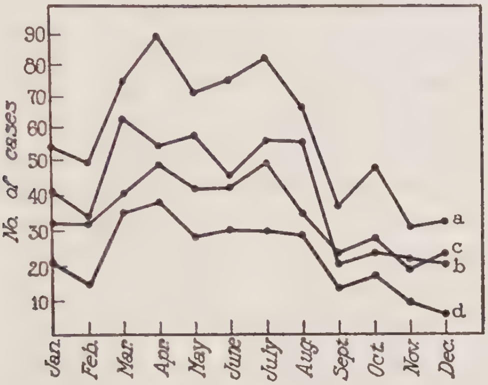

FIG. 2. SEASONAL DISTRIBUTION OF DIPHYLLOBOTHRIUM ANEMIA AND CRYPTOGENETIC PERNICIOUS ANEMIA (LINDSTRÖM (242))

a, both types combined (Lindström); *b*, both types combined (Schauman and Saltzman); *c*, cryptogenetic pernicious anemia (Lindström), and *d*, Diphyllobothrium anemia (Lindström).

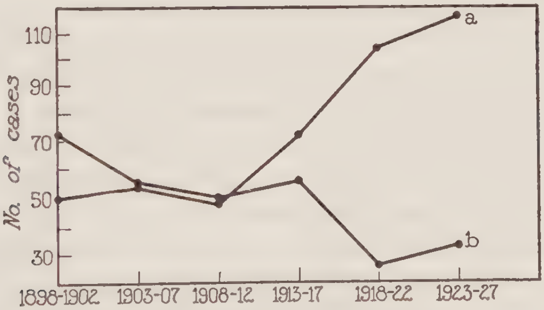

FIG. 3. MORBIDITY TENDENCY IN DIPHYLLOBOTHRIUM ANEMIA AND CRYPTOGENETIC PERNICIOUS ANEMIA CALCULATED IN FIVE-YEAR PERIODS (LINDSTRÖM (242))

a, cryptogenetic pernicious anemia, and *b*, Diphyllobothrium anemia with that of Victor Schilling which was built from 1595 cases of cryptogenetic pernicious anemia in Germany. The decline in Diphyllobothrium anemia may be accounted for by several facts: the disease gradually has become better known and earlier recognized; the patients have become educated to employ medical aid in the early stages of the disease, which enables them to be taken care of by the home physician without hospitalization. On the other hand no mention is made of changes in dietary habits which may have cut down the frequency of infestation.

Constitution and predisposition. Whatever may be said regarding the influencing factors that have been mentioned, it seems obvious that they constitute auxiliary, exogenous factors only. Even the fish tapeworm cannot alone produce the anemia. In Diphyllobothrium anemia a cryptogenetic etiologic factor is present, and probably is the same as that which is the underlying factor in cryptogenetic pernicious anemia (361, 383–386, 389).

There are certain indications for the existence of predisposition in persons in whom anemia develops. It has been observed that a person who has gone through Diphyllobothrium anemia at one time may later become reinfested with the tapeworm without the development of anemia (388). Nevertheless, Reyher has recorded one case; Schauman (382), three cases, and Lindström (242) has reviewed four cases of recurring Diphyllobothrium anemia. The interval between two attacks varied between eight months and seven and a third years. All of the patients were cured each time. However, the possibility that the same parasites remained during the free intervals, despite negative examinations of stools after a vermifuge had been given, cannot be excluded in all instances with certainty.

Schauman (383), Rosenqvist (354), Holsti (185), Sahli (369) and Schauman and Saltzman have observed that a patient who has gone through typical Diphyllobothrium anemia successfully at one time, on a later occasion may succumb to typical cryptogenetic pernicious anemia without the presence of intestinal parasites. Lindström (242) has compiled seventeen cases of this kind (table 7). Six of the patients were men and eleven were women. Of patients with Diphyllobothrium anemia cryptogenetic pernicious anemia developed later in life in 5.7 per cent, or, of the patients who were treated for cryptogenetic pernicious anemia, 3.8 per cent had previously had Diphyllobothrium anemia. These data were derived from hospital records in Helsingfors. The recidivation occurred in eight cases in the second year, in two cases in the third year, in two cases in the fourth year, in one case in the fifth, eighth and tenth years, and in two cases in the thirteenth year. All decades of life between the ages of twenty and seventy years and more were almost equally represented.

Tallqvist (433) has briefly referred to a patient who at one time was cured of typical Diphyllobothrium anemia and later fell victim of typical aplastic anemia in the absence of intestinal parasitism.

In one instance Schauman and Saltzman had occasion to diagnose chronic hemolytic icterus. The patient was a man aged thirty-two years, who sixteen years previously had had a typical attack of Diphyllobothrium anemia and had been cured by vermifuge. Further investigation revealed that a nine-year-old daughter also had chronic hemolytic jaundice.

TABLE 7

Sex and age distribution of patients with Diphyllobothrium anemia who later succumbed to cryptogenetic pernicious anemia (Lindström) (242)

AGE, YEARS	20–29	30–39	40–49	50–59	60–69	70 AND MORE
Males	2	1	1	1	1	
Females	1	3	2	2	2	1
Total	3	4	3	3	3	1

In the anamnesis of some of the female patients suffering from Diphyllobothrium anemia chlorosis has been noted. In some instances, the anemia associated with the fish tapeworm has been classified as simple, or secondary, in type. Botkin (56) reported grave chlorosis in a young girl who harbored the worm and who recovered fully following administration of vermifuge. Schauman (381) reported three cases of chlorosis in young girls with ova of Diphyllobothrium in the stools. One case was that of a housemaid aged twenty-one years in which erythrocytes numbered 2,300,000 in each cubic millimeter of blood, the concentration of hemoglobin was 22 per cent, and the color index was 0.56. The second case, also, was found in a housemaid, aged eighteen years; erythrocytes numbered 3,100,000; the concentration of hemoglobin was 29 per cent and the color index was 0.56. The third case occurred in a farmer's daughter, aged twenty-two years; erythrocytes numbered 4,800,000, the concentration of hemoglobin was 54 per cent, and the color index was 0.66. Schauman did not include these cases in his material, but considered them to be cases of chlorosis in carriers of Diphyllobothrium. He did not claim that there was any causal connection between the two conditions. One of these patients recovered fully from the chlorosis on treatment with iron, without removal of the worm. Ewald (128) and Cramer, only, reported a color index below 0.7 (0.33 and 0.32 respectively), but otherwise their cases had all the appearances of cases of pernicious anemia. Cramer's patient died. Meyer (272), Savitz and Rosenthal recorded cases in which the color index ranged between 0.7+ and 0.76. Ewald's, Meyer's and Rosenthal's patients recovered promptly following administration of vermifuge, whereas no mention was made of the outcome in Savitz' case. Warthin (464) stated that in all the cases of infestation with Diphyllobothrium which came to the attention of the staff of the Pathologic Laboratory of the University of Michigan, there was clinical evidence of more or less severe anemia, usually of a secondary type. The recorded cases of simple anemia associated with *Diphyllobothrium latum* are too few and too incomplete to warrant definite deductions.

How do these facts compare with conditions found to have a bearing on pernicious anemia? Schauman and Saltzman, Conner (92) and others maintain that pernicious anemia may assume a more or less aplastic character during periods of its course and especially in the terminal stages. Tallqvist's case is therefore in harmony with the present contention concerning pernicious anemia. This phase of the subject, however, will be more fully considered later.

As far as I have been able to ascertain, no instance has been recorded of pernicious anemia and hemolytic icterus in the same person at the same or at different times. Conner (92) has informed me, however, that occasionally one may run across a case in which it is extremely difficult to make a differential diagnosis of pernicious anemia and hemolytic icterus at a certain time in the course of the disease. This may be of significance in the evaluation of Schauman's interesting case.

Recently secondary forms of anemia, of known and of unknown etiology, have been subjected to intensive studies. Evidence has been offered to show definite relationship between cryptogenetic secondary anemia and cryptogenetic pernicious anemia. Schauman and Saltzman recorded in detail the case of a woman who had been treated for chlorosis at the age of sixteen years, for cryptogenetic pernicious anemia at the age of twenty-eight years, and who presented at the age of thirty-three years, a rather typical picture of cryptogenetic secondary anemia. Transition of a secondary type of anemia into a pernicious type has been observed by Neuburger, Thaysen, and Schauman (386). This possibly explains the few instances of simple anemia in Diphyllobothrium carriers. Such cases may have been observed in the course of a phase of temporary "secondary blood reaction," if use of such an expression is permissible. Or the person afflicted may have responded more readily with that mode of reactability and may have been unable to produce the "pernicious blood reaction."

The question naturally arises whether there exists a familial tendency to the development of Diphyllobothrium anemia. Schauman (385) succeeded in personally investigating fifteen families in which two or more members of each had or had had Diphyllobothrium anemia. Cramer's report of simultaneous occurrence of anemia in three sisters of about the same age (thirty-five, thirty-six and thirty-eight years) is particularly interesting in this connection. The coexistence of this anemia in one member of the family, and of cryptogenetic pernicious anemia in another was furthermore observed by Schauman (386) in two families and by Tallqvist (431), and Isaacs, Sturgis and Smith, each in one family. In five families Diphyllobothrium anemia was seen in one member and chlorosis without tapeworm in one or two sisters in the same family (389). In Cramer's family the youngest of the three sisters had a very low color index but otherwise presented features consistent with pernicious anemia.

Regarding cryptogenetic pernicious anemia, I may briefly refer to its familial occurrence (389) as a well established fact. It is also well known that cryptogenetic pernicious anemia has been seen in families of which other members have shown definite symptoms of simple anemia (91, 132, 156, 294, 319).

Several of these observations point toward a similar, inheritable, constitutional predisposition in Diphyllobothrium anemia and cryptogenetic pernicious anemia, and also toward a close inner resemblance between the two. In all familial instances the relationship between the patients has been close, that is, a blood-relationship: children of the same parents, child and parent, nephew and uncle, nephew and aunt, niece and aunt.

It is a problem, as yet unsettled, whether we are dealing with either a recessive or dominant hereditary factor or with a mutation, although Mustelin, who observed cryptogenetic pernicious anemia in grandmother, mother and daughter of the same family, feels more inclined to consider it dominant.

The common occurrence of alcoholism and lack of self control in the patient's father has been emphasized as having some significance when the familial constitutional make-up is being considered. A more outstanding feature, however, is the more or less pronounced nervous and mental disturbances, mainly of a functional order: neurotic and psychoneurotic conditions, either in the patient or in some near relative. Ketcher described a case in a woman of subnormal mentality; and an inferior intellectual level was obviously present also in the three sisters noted by Cramer. Schauman (381) saw Diphyllobothrium anemia in a woman who had suffered from epileptic attacks for years before the onset of anemia, and Schauman and Saltzman saw epilepsy develop after full recovery from Diphyllobothrium anemia.

These nervous and mental manifestations are not a result of the anemia. They may occur before onset of the anemia, during its course, or even after cure has been accomplished. Rather one might say that these disturbances are seen to occur in persons who have had, have, or will have Diphyllobothrium anemia. In other words, they strengthen the contention that the basis of the anemia is constitutional.

Symptoms. The onset, development, and course of Diphyllobothrium anemia is clinically characteristic, and the blood picture, morphologically typical.

The onset is generally insidious. So gradually do the symptoms appear that the patient may be unable to give definite information as to the time of onset. In most instances, pallor and weakness develop without any known causal condition. In some cases, the condition seems to have been ushered in by a state of lowered general resistance such as is seen following an infectious disease or a mental upset.

Schauman and Saltzman referred to two instances in which mental strain seems to have precipitated the onset of the anemia. One patient was a government official of high rank, who had a great deal of annoyance and worry associated with his work; the other was a woman cashier who had embezzled and suffered greatly under the consequences of her crime. Vermifuge cured the anemia in both cases.

That physical and nervous exhaustion and mental excitement are of significance also in the development of cryptogenetic pernicious anemia has been shown by Grawitz (160), Cabot (76), Schauman and Saltzman, and others.

Information concerning early symptoms is not adequate. One early symptom, however, has attained a prominent place, although its cause and significance still are uncertain. I refer to the stomatitis-glossitis. This symptom may precede the anemia for years, and may be present from time to time in Diphyllobothrium carriers in whom anemia does not develop, as has already been mentioned (25, 86). Unfortunately, in many reports of cases information is lacking as to the presence or absence of this symptom, so that the frequency of its occurrence is difficult to determine. Schauman (381) mentioned its presence in seven (19.44 per cent) of the thirty-two cases he himself observed and recorded in his first monograph but he did not make any statement as to its absence in the remaining cases. Schauman and Saltzman stated that it was noted in the majority of the cases, but did not indicate any difference between the frequency in Diphyllobothrium anemia and cryptogenetic pernicious anemia. Becker (29), Isaacs, Sturgis and Smith, and Saltzman (374) mentioned it in their reports of cases accompanied by anemia. Systematic questioning of the patients brought out that ten of Becker's thirteen patients (77 per cent) had, or had had, one or more periods of stomatitis-glossitis. This gives at least a strong indication that the condition is not infrequent in tapeworm anemia. Sometimes the symptoms may be the outstanding complaint which brings the patient to the physician; but in most instances they are not so pronounced. Often the symptoms are so mild and occur at such long intervals that the patients can remember them only when they are closely questioned.

Schauman (386) emphasized dental sepsis and carious teeth as an important part of the oral sepsis.

The appetite is generally impaired; only in exceptional cases is it increased above normal. Gastro-intestinal irregularities, such as have been described in nonanemic carriers may or may not appear. Becker (27) mentioned the rather frequent occurrence of a bad taste in the mouth. The disturbances in the gastro-intestinal tract may be so severe as to constitute the reason for medical consultation and the anemia may be discovered only incidentally by the physician.

Aside from this feature, the early symptoms of Diphyllobothrium anemia are similar to those of any type of anemia, as far as anamnesis makes them evident. The patient becomes more easily fatigued by physicial exertion. Soon, definite pallor becomes increasingly noticeable. Physical strength and vigor gradually decrease. Dizziness, tinnitus, and throbbing or aching in the head make their appearance. Moderate exertion causes palpitation of the heart, tachycardia, and dyspnea. Occasionally, edema is noticed.

The symptoms, although they may be spontaneously arrested at any point of their development, have a tendency to become gradually and progressively worse. Usually the weakness is the predominating feature, but occasionally one or another of the symptoms related may be in the foreground.

When the patient becomes incapacitated and is forced to go to bed, the anemia will be of varying intensity, and often the number of erythrocytes will be as low as 1,000,000 in each cubic millimeter of blood. It is astounding to note how long some patients may keep themselves up and may perform even heavy physical labor. Schauman and Saltzman once saw a man whose erythrocytes, on his admission to the hospital, numbered only 621,000 in each cubic millimeter of blood; nevertheless until five days before, he had kept at his work of machinist in a sawmill. The fact that the onset is so insidious and that the patient comes to the physician so late in the development of the anemia is the principle reason why it is seldom studied in its early stages.

In no essential way does Diphyllobothrium anemia in its early stages depart from the course of cryptogenetic pernicious anemia or of any other severe type of anemia.

In fully developed anemia the patient's appearance is essentially identical with that of a patient suffering from cryptogenetic pernicious anemia (342, 363–366, 380).

The facial expression may in advanced cases bear witness of depression and great weakness. Mental activities, like thinking and speaking, may be noticeably retarded. Sometimes an irritable, sulky or cross disposition is observed.

The skin is pale with a waxy yellowish tinge, which in certain instances may be so pronounced as to suggest jaundice. This simulation is strengthened by the occasional yellowish discoloration of the sclerae. In a number of cases, this yellow discoloration of the skin is lacking, and a grayish tint, or, due to higher concentration of pigment, a brownish tint takes its place (178). There is no definite relationship between the severity of the anemia and the degree of cutaneous pigmentation, for there are early cases in which there is much pigment, and advanced cases in which it is absent. The basis for this change in color probably is the presence of increased concentration of serum bilirubin, due to a higher rate of destruction of erythrocytes. The variability of the shades of color probably is determined by the prevailing degree of destruction of erythrocytes. Following successful treatment with an anthelmintic, this yellowish tinge, as a rule, disappears rapidly. There are cases, however, in which the color clears up, but the improvement is retarded or does not materialize. This may be explained on the basis that the destructive mechanism is halted but that sufficient regenerative power is absent.

The skin is generally dry and lusterless, but otherwise remarkably void of pathologic changes. The normal elasticity is maintained.

An edematous state, which is found somewhat less frequently in Diphyllobothrium anemia (50 per cent) than in cryptogenetic pernicious anemia is evidenced by a certain puffiness of the face, and slight swelling about the ankles, and in the feet and hands (11, 14, 48, 90, 97, 164, 178, 203, 211, 238, 272, 300, 354, 379, 380, 435, 450). Herzog also could demonstrate a slight amount of ascites in his case.

A hemorrhagic diathesis is present in about 50 per cent of the cases. Usually it is not pronounced. It occurs at the height of the disease. Small petechial and ecchymotic spots are scattered over the trunk and extremities, almost always on the basis of an edematous condition of the skin. In advanced cases, transient blurring of the vision is a common complaint, and retinal hemorrhage is frequently observed (87, 90, 299, 354). More extensive subconjunctival effusion has been observed in a few instances. Rather extensive submucous suggillation of the tongue was observed in one case (381). Epistaxis (67, 96, 164, 300, 327, 380, 435), bleeding from the gums (178, 354, 380) and melena (450, 478) are other signs of the same tendency, and hemoptysis, without demonstrable pulmonic pathologic change, occurred in one of Schauman's (381) cases.

Metabolism is sometimes altered. The subcutaneous fat is often moderately reduced. However, in many instances it is remarkably well preserved, even abundant. Real cachexia rarely develops. The cause of this notable preservation of the nutritional state is not readily found. The basal metabolic rate has been determined in a series of cases (389) and has been compared with that of patients with cryptogenetic pernicious anemia and with that of normal controls. All three series were essentially alike.

The metabolism of protein has been thoroughly investigated by Rosenqvist (353, 354). From his experiments, he concluded that in Diphyllobothrium anemia, as well as in cryptogenetic pernicious anemia, periods of increased catabolism of protein alternate with periods of storing of nitrogen. In Diphyllobothrium anemia he could demonstrate that, before expulsion of the parasites, and on entirely indifferent treatment, not only could a nitrogen balance be maintained, but frequently storage of nitrogen could be observed. He also found that the loss of protein often was too great to be accounted for by destruction of erythrocytes only, and therefore he assumed that the excess protein was derived from other tissues. In certain cases he made the remarkable observation that the blood picture might improve in spite of continued negative nitrogen balance. Therefore, no definite parallelism could be demonstrated between alterations in the nitrogen equilibrium on one side and the changes in the condition of the blood on the other. Rosenqvist concluded that the catabolism of protein was dependent on direct toxic action and not on the anemia as such; the supposed toxin injured not only erythrocytes but other cells as well.

The temperature of the body is elevated in the majority of cases (85.6 per cent). The fever usually appears periodically and the temperature reaches a moderate level. For the most part, it does not adhere to any particular type, although it is sometimes remittent. Between the fever and the degree of anemia no parallelism can be established, nor could Rosenqvist (354) find any relationship between fever and catabolism of protein. Usually, however, the higher rises of the temperature are associated with aggravation of the general condition and of the abnormality of the blood picture. In some of the fatal cases, there is continued fever up to the time of death, and in others, marked drop in the temperature to subnormal levels preceding death.

Sometimes a temperature that has been elevated rapidly returns to normal following expulsion of the worm. Frequently, however, a temperature that has been elevated climbs to higher levels for several days after expulsion of the worm, before it goes back to normal. Occasionally one or more periods of elevated temperature are observed for days or weeks after subsidence of the fever, following expulsion of the worm. These rises are usually slight, but the temperature may go to 40.2°C. When the temperature rises to great height the general condition of the patient usually is rather low, and the blood picture may change decidedly for the worse. In those cases in which fever persists for a long time following expulsion of the worm, the prognosis should be made guardedly. Certain cases are observed in which the temperature rises following expulsion of the worm, although fever did not exist before. Then there are other cases in which the fever disappears before the worm has been expelled. Finally, in a large series of cases, there is no significant elevation of the temperature either before or after the worm has been expelled; in others slight rise appears to be unaffected by expulsion of the worm. The impression is gained, therefore, that it is not elimination of the parasite as such which determines the temperature after the worm has been expelled.

The question of the cause of the fever is still unsettled. Destruction of erythrocytes hardly can be held responsible, for fever has been observed during periods of regeneration and also has not been observed in the presence of positive urobilin and urobilinogen reactions of the urine. There is no evidence that the temperature is of infectious origin. It is possible that the cause lies in processes of immunization. What has been said about the type of fever in Diphyllobothrium anemia can be applied equally well to cryptogenetic pernicious anemia.

Symptoms referable to the respiratory tract have no more significance in Diphyllobothrium anemia than they have in cryptogenetic pernicious anemia and such complications are rarely seen. Dyspnea, on account of diminished capacity of the blood to carry oxygen, is a common complaint and is common to all forms of anemia when they have reached a certain intensity. At first it occurs only on exertion, later, it appears on ordinary moving about, and in the most severe cases, even on complete rest in bed. The rate of respiration may be increased, or may be about normal, but in the latter case the respiration is deeper and fuller, sometimes markedly so. The deep, slow effortless breathing (die grosse Atmung) is a bad sign (389) whereas a rapid respiratory rate does not seem to have much prognostic significance. Schauman and Saltzman think it is not at all improbable that toxic action is the underlying cause of "die grosse Atmung," perhaps acting through special metabolic disturbance or some other factor.

Symptoms referable to the cardiovascular apparatus are prominent in Diphyllobothrium anemia, as they are in other types of severe anemia. Palpitation of the heart and increased pulse rate are closely associated with the dyspnea, as has been mentioned. In the most severe cases, the cardiac impulse is felt and is seen to the left of the midclavicular line, for the most part, in the fifth intercostal space. Not seldom, this impulse is rather diffuse. Pulsation frequently is seen in the blood vessels of the neck, sometimes in the suprasternal notch and epigastrium. The area of absolute cardiac dulness is increased in many instances, more frequently to the right, and is indicative of cardiac dilatation. The most common symptom that is traceable to the cardiovascular system is a functional, systolic, "hemic" murmur. It was not present in a group of cases in which the intensity of the anemia ranged from moderate to a condition in which erythrocytes numbered 395,000 in each cubic millimeter of blood. Consequently, the murmur is not directly a result of the anemia only, but some other, unknown factor must play a part. A systolic murmur may sometimes be produced in the peripheral arteries by exerting slight pressure on them with the stethoscope. A venous hum, Nonnengeräusch, heard over the jugular veins is rarely absent; it is especially clear when the patient is in a sitting or standing position. Schauman found it present in thirty-four of thirty-six cases particularly examined for this symptom. In certain cases it could be found only on the right side; in others, it was definitely more pronounced on the right side than on the left.

Venous pulsation may sometimes be observed. The hum will often make itself known as fremitus to the palpating finger.

The digestive tract is regularly involved. Dental sepsis was recorded in the cases of Savitz and Lind (240), and caries dentium by Schauman (386). Stomatitis and glossitis has been referred to by Becker (26, 29), Minot (285), Schauman and Saltzman, Saltzman (373, 374), and others. It is identical with that so often seen in carriers without anemia, and repetition of its description is therefore unnecessary. Earlier reports of cases are negligent in their dealing with pathologic changes of the oral mucosa and infrequent mention of them does not indicate their rarity. Later reports of series of cases demonstrate their preponderance.

Stomatitis and glossitis in nonanemic (86) as well as in anemic (389) carriers is in every respect identical clinically with the condition Hunter (190) described and emphasized as being present in the cryptogenetic form of pernicious anemia. It is considered a symptom of the toxic state that supposedly underlies the syndrome of pernicious anemia. The assumed Diphyllobothrium toxin seems to have a particularly strong elective affinity for the digestive tract.

If there is any difference in the stomatitis and glossitis in Diphyllobothrium anemia as compared with that in cryptogenetic pernicious anemia, the symptoms may perhaps be slightly less marked in the former (26).

Even in the absence of oral pathologic change and its direct influence on intake of food, the appetite often is considerably diminished, as reported by Winiarski, Minnich, Askanazy, Hagelstam, Sievers and von Bonsdorff, Neubecker, Bard, Rosenqvist (354), Nicholson, and others. This loss of appetite may be either selective, directed against certain kinds of food, or it may apply to all foods. Sometimes the aversion to food is so strong that even sight of it causes nausea. Vomiting as a symptom belongs to the more advanced stages and has been noted by Schapiro, Biruli, Neubecker, Orlowsky, Tonnel, Rosenqvist (354), Meyer (272), McGaffin, Haden, Grünmandel, Becker (29), and others. Dyspepsia was present in the cases recorded by Minnich, Bruhn-Fåhraeus, Courmont and André, Rosenqvist (354), Becker (29), Nicholson and others. Sievers and von Bonsdorff observed epigastric distention and colicky pains in their case. Richter, Maurer and Eyl noted, in their patient, pain and tenderness in the right lower abdominal quadrant, which simulated appendicitis. Most common among intestinal symptoms, however, is diarrhea, which may be periodic or continued, mild or severe (14, 29, 90, 114, 164, 197, 238, 268, 302, 310, 354, 360, 410, 450, 464, 478). The periodic diarrhea may alternate with constipation as Schapiro, Podwissotzky, Biruli, Bruhn-Fåhraeus, Kurimoto, Tonnel, Rosenqvist (354), and others have seen. In a certain number of cases there has been more or less persistent constipation (11, 284, 300, 354).

Thirst may be definitely increased.

Gastric function has been thoroughly studied by several investigators; on the other hand, mention of this important point unfortunately is missing in many reports of cases. The secretory function is of special interest, so far as there is insufficiency of the secretion of hydrochloric acid in the majority of the cases. In carriers without anemia gastric acidity is normal, as has been shown. In Diphyllobothrium anemia the occurrence of achlorhydria is much higher than it is in normal persons, but evidently it is not quite so high as that found in cryptogenetic pernicious anemia. Askanazy, Bruhn-Fåhraeus, Bard, Rosenqvist (354), Sahli (369), Schmidt, Faber (131), Schauman and Levander, Grünmandel, Becker (26, 29), Lind (240), Isaacs, Sturgis and Smith, Saltzman (373, 374) and Belonogowa have reported cases with achlorhydria. Since Schauman (381), Rosenqvist and Becker have shown that hydrochloric acid may reappear in the gastric secretion after cure of the anemia, those cases in which analysis of gastric content has not been done at the height of the anemia, and in which, at some later date, free hydrochloric acid has appeared in the gastric content, have not been considered in this study in arriving at a conclusion as to the frequency of the presence or absence of hydrochloric acid in the gastric content. But, those cases in which achlorhydria has been present within seven weeks of beginning recovery from anemia have been included, since there is every probability that free hydrochloric acid was lacking at the height of the anemia as well.

Of eighty-seven cases in which estimations of acidity in the gastric content were carried out, achlorhydria was present in seventy-three (83.9 per cent) and free hydrochloric acid in fourteen (16.1 per cent). Of the latter group no cases are reported with hydrochloric acid higher than 20 (end point in 10 cc. of gastric content produced by 2.0 cc. of tenth-normal sodium hydroxide). Rosenthal stated that in his case gastric acidity was normal. In Schauman's (381) series of eleven cases, reported in 1894, free hydrochloric acid was present in only one, and in this case there was definite hypochlorhydria after Ewald's test meal; the reaction with Congo red as an indicator was negative once and slightly positive another time; after Leuckhard's test meal the reaction with Congo red was positive. Rosenqvist (354), in his series of fifteen cases, found achlorhydria in twelve and free hydrochloric acid in three; the reaction with Congo red was positive in one case, slightly positive in another, and the concentration of hydrochloric acid was determined to be 15 in the third case. Schauman and Levander reported on twenty-nine cases in 1917, in five of which free hydrochloric acid was present, but in subnormal amount; the highest reading for hydrochloric acid was 18. Of Becker's (29) twelve cases, free hydrochloric acid was present only once; in this case, the free hydrochloric acid was 20 and the total acidity, 30. In the rest of the cases, no free hydrochloric acid was present. Saltzman (373, 374) found achlorhydria in all of his five cases.

The sex of the patient has been given in eighty-four cases; forty were males and forty-four, females. Of the patients with achlorhydria, thirty-three were males and thirty-eight were females. Of those with free hydrochloric acid, seven were males and six were females. There is apparently no predilection for either sex in either group.

The patient's age was given in eighty-six cases. The distribution of the cases in the various age groups is seen in table 8. The last column indicates the influence of age on the gastric secretion of hydrochloric acid in Diphyllobothrium anemia. There is a definite increase of preserved secretory function in the lower age groups.

In some cases, as had been said, in which achlorhydria was present, at the height of the anemia, free hydrochloric acid was found to be present later. Unfortunately only a few cases have been investigated in this regard and it is not known, therefore, in how great percentage resumption of the gastric secretory function takes place. I have found only six cases in which this is reported (table 9) and in these cases the acidity was determined as follows: in three of them the reaction with

Congo red was positive; in one the concentration of the free hydrochloric acid was 24, in one 30, and in one 38.

Schauman and Levander undertook investigation of the secretory function in twenty-two cases in which, at some earlier time, Diphyllobothrium anemia had been present and cure had been effected. The period elapsed from the time of the attack of anemia to the time of the reëxamination ranged from twenty-four to two years. In this series, they found eleven patients without, and eleven with, free hydrochloric acid; the latter cases are listed in table 10. The patients were all in fair physical condition, although nine had general nervousness, one was imbecile, one had maniac depressive psychosis, five were victims of alcoholism, and one had renal tuberculosis. The condition of their blood at the time of the examination was as follows: the concentration of hemoglobin ranged from 73 to 104 per cent and erythrocytes numbered from 4,000,000 to 5,770,000. Ova of *Diphyllobothrium latum* were found in three cases in none of which there was any sign of anemia at the time of reëxamination. Whereas only 16.1 per cent of the patients with Diphyllobothrium anemia had free hydrochloric acid in the gastric content at the height of the disease, in 50 per cent free hydrochloric acid was present at a certain time after recovery had taken place. In the remainder, functional recovery apparently has taken place. The finding of free hydrochloric acid at a later date is dependent on presence or absence of free hydrochloric acid previous

TABLE 8

Age distribution of gastric acidity in Diphyllobothrium anemia

AGE	HYDROCHLORIC ACID		ACHLORHYDRIA
	Present	Absent	
years			*per cent*
0–20	6	7	53.85
21–30	4	11	73.33
31–40	2	18	90.00
41–50	1	19	95.00
51–60	1	11	91.67
61–70		6	100.00
Total............	14	72	83.72

TABLE 9

Reëxamination to determine gastric acidity in cases of achlorhydria associated with Diphyllobothrium anemia

INVESTIGATORS	SEX	AGE AT TIME OF ANEMIA *years*	TIME LAPSED SINCE RECOVERY FROM ANEMIA	GASTRIC ACIDITY	INVESTIGATORS WHO MADE REËXAMINATION
Schauman (381)	F.	37	23 years	Hydrochloric acid absent	Schauman and Levander
Schauman	M.	17	1 month	Hydrochloric acid present (test with Congo red and litmus positive)	Schauman (381)
Schauman	F.	54	21 years	Hydrochloric acid present (test with Congo red slightly positive with litmus positive)	Schauman and Levander
Schauman	F.	38	21 years	Hydrochloric acid = 0; total acidity = 42	Schauman and Levander
Schauman	M.	12	18 years	Hydrochloric acid = 30; total acidity = 57	Schauman and Levander
Rosenqvist (354)	M.	38	2.5 months	Hydrochloric acid absent	Rosenqvist (354)
Rosenqvist	F.	19	1.5 months	Hydrochloric acid absent	Rosenqvist
Rosenqvist	F.	28	11 months	Hydrochloric acid absent	Rosenqvist
Rosenqvist	M.	40	1 month	Hydrochloric acid absent	Rosenqvist
Rosenqvist	F.	39	7 weeks	Hydrochloric acid absent	Rosenqvist
Rosenqvist	M.	53	3 weeks	Hydrochloric acid absent	Rosenqvist
Rosenqvist	M.	46	6 weeks	Hydrochloric acid = 38; total acidity = 58	Rosenqvist
Rosenqvist	F.	36	1 year	Hydrochloric acid = 24; total acidity = 56	Rosenqvist
Becker (29)	F.	40	7 weeks	Hydrochloric acid present (test with Congo red positive)	Becker (29)

to the attack of anemia and, possibly, on duration of the anemia. Vesa (449a) has reported two cases of Diphyllobothrium anemia with achlorhydria which were treated with liver in the presence of the tapeworm; in these cases free hydrochloric acid reappeared in the gastric juice before removal of the worm. Shaw and Hurst report the only cases of which I have record, in which there has been apparent recovery from cryptogenetic pernicious anemia associated with return of gastric secretory function. It should be noted that dyspeptic symptoms often are absent, despite lack of hydrochloric acid. What, then, is the relationship between achlorhydria and anemia? There are three possibilities: 1. Achlorhydria may be the cause of anemia. The tapeworm as such does not seem to influence directly the gastric secretion, not even when anemia develops. In a certain number of cases, free hydrochloric acid is present at the height of the anemia; therefore, one cannot say that the anemia, in all instances, is caused by the achlorhydria. 2. Anemia may be the cause of achlorhydria; functional recovery of gastric secretion of hydrochloric acid following cure of the

TABLE 10

Examination to determine gastric acidity in cases of Diphyllobothrium anemia in which gastric acidity was unknown at the time of the anemia

EXAMINER	AGE	SEX	TIME LAPSED SINCE RECOVERY FROM ANEMIA	GASTRIC ACIDITY	
				Hydrochloric acid	Total acidity
	years				
Rosenqvist (354)	25	M.	3 weeks	48	84
Rosenqvist	23	M.	3 weeks after beginning recovery	56 (34)	100 (66)
Rosenqvist	21	F.	3 months	60	84
Rosenqvist	50	M.	2 years	22	40
Schauman and Levander	18	F.	2 years	90	151
Schauman and Levander	23	M.	7 years	46	58
Schauman and Levander	25	M.	9 years	10	43
Schauman and Levander	47	F.	9 years	19	28
Schauman and Levander	19	F.	11 years	5	33
Schauman and Levander	53	M.	18 years	19	76
Schauman and Levander	18	M.	24 years	62	105

anemia by vermifuge is supporting evidence of this. But many patients have achlorhydria before development of the anemia. Therefore, achlorhydria cannot always be the result of the anemia. 3. The same cause may be underlying both conditions. Schauman and his followers suspect that the predisposition for achlorhydria simply is a partial symptom of the constitutional condition which affects persons in whom Diphyllobothrium anemia or cryptogenetic pernicious anemia develops. This is in line with Meulengracht's and Conner's (91) observation of a high incidence of achlorhydria in relatives of patients with cryptogenetic pernicious anemia. In those cases, however, in which the gastric secretion appears normal at the onset of the anemia, one may assume that this function may be lost in the course of protracted anemia. The fact that in Diphyllobothrium anemia an etiologic factor can be removed, may explain why achlorhydria is seen less often and less persistently in Diphyllobothrium anemia than in cryptogenetic pernicious anemia. In the latter type only exceptional cases with presence of free hydrochloric acid have been recorded, by Cabot (76), Faber and Gram, Schauman and Levander, and others. It must also be borne in mind that in the majority of cases of Diphyllobothrium anemia fractional analysis of gastric content after a test meal was not done, and to my knowledge in no instance was the alcohol test meal or the method employed of stimulating gastric secretion by administration of histamine.

The motor function of the stomach, in many instances, is speeded up so that gastric content cannot be obtained by means of the stomach tube forty-five minutes to one hour after the test meal has been ingested. Frequently, therefore, the test meal must be aspirated as early as half an hour after it has been taken. This feature has been noted also when achlorhydria is associated with other pathologic conditions, such as exophthalmic goiter, and therefore it may be always associated with functional achlorhydria.

Becker (29), in 1926, examined the gastric content for *Escherichia coli* in a large series of cases, among them thirteen cases of Diphyllobothrium anemia and eighteen cases of cryptogenetic pernicious anemia. He was led to this study by certain established facts: (1) *Escherichia coli* is found in the small intestines and in the stomachs of patients with cryptogenetic pernicious anemia; (2) *Escherichia coli* yields an extract which, when injected into experimental animals, may produce hyperchromatic anemia, and (3) experimental intestinal strictures may give rise to development of pernicious anemia. When this occurred, *Escherichia coli* is found in the intestinal content above the strictures, whereas in the animals in which pernicious anemia does not develop, *Escherichia coli* is absent, likewise, above the strictures.

Becker's observations may be summarized as follows: 1. *Escherichia coli* is present in stomachs of some patients with achlorhydria but almost never in others; however, stomachs of patients with achlorhydria do not always contain *Escherichia coli.* 2. *Escherichia coli* in the gastric content is found in cases of cryptogenetic pernicious anemia regularly and in Diphyllobothrium anemia frequently; occasionally it is found in Diphyllobothrium carriers without anemia and in certain other conditions. 3. In cryptogenetic pernicious anemia, *Escherichia coli* does not disappear from the stomach during remissions. In Diphyllobothrium anemia, however, there seems to be disappearance, or at least a decrease in number after recovery. 4. Indicanuria is more commonly found when *Escherichia coli* is present in the gastric content; when *Escherichia coli* is absent in cases of Diphyllobothrium anemia there is no indicanuria; in two instances, the indican reaction was negative in the presence of *Escherichia coli* in the stomach.

Unless the patient already has undergone successful treatment for expulsion of the worm, ova of *Diphyllobothrium latum* will almost always be detected in the stools. During certain periods, however, ova may not be found on repeated examinations, only later to appear in abundance.

The lymphatic system is not involved in the clean-cut case of Diphyllobothrium anemia. One sees occasionally, especially in younger persons, hypertrophic tonsils and slight adenopathy in the neck, but this is to be considered rather an expression of a constitutional make-up than a symptom of the disease.

The skeletal system presents localized tenderness to pressure in a great number of cases (354). This is generally most marked on the sternum, particularly the lower end, and, less frequently, on the humerus, tibia and femur.

Although the output of urine, at the height of the disease, may be normal, increased or diminished, in the convalescence from Diphyllobothrium anemia and in remissions of cryptogenetic pernicious anemia, there is usually more or less marked polyuria. This polyuria cannot be accounted for by absorption of edema fluid, because the edema usually is slight and because the polyuria continues for a considerable time after the last trace of edema has disappeared and even after the blood picture has returned to normal. The specific gravity often fluctuates in a corresponding manner. When the urine is secreted in relatively small quantities the specific gravity frequently is high. In cases in which the output of urine is greater it is sometimes normal, sometimes lowered. Case 1, in table 11, exemplifies this. It seems reasonable to assume that this relative polyuria, in some instances is secondary to a correspondingly increased intake of water, whether this is accompanied by thirst or not. But in an instance such as that exemplified by case 2 (table 11), there seems to be present a certain degree of hyposthenuria, a diminished power of the kidneys to concentrate the urine. Saltzman (389) found that such persons after recovering from Diphyllobothrium anemia would have essentially normal power of dilution and concentration of the urine.

TABLE 11

Daily output and specific gravity of urine averaged for consecutive periods of ten days each, in two illustrative cases (after Schauman and Saltzman)

CASE		PERIOD						
		1	2	3	4	5	6	7
1	Urinary output, cc. Specific gravity	950 1.023	1,120 1.025	1,380 1.023	1,580 1.023	1,510 1.022	2,070 1.015	2,240 1.017
2	Urinary output, cc. Specific gravity	1,650 1.015	1,800 1.014	2,290 1.014	2,200 1.014	1,782 1.016		

The reaction of the urine is usually acid. The color is frequently rather dark, even in some of those cases in which the output is high.

The tests for urobilin and urobilinogen in the urine are sometimes positive (162, 164, 178, 351), and sometimes negative. Not seldom they are negative in advanced cases. Periods of positive reaction may alternate with periods of negative reaction in the course of the disease in the same patient. The lessening and disappearance of the urobilinuria after expulsion of the worm is rather striking. Occasionally, however, the reaction will remain positive for some time after the worm has been removed even when regeneration of the blood has progressed for a while. Nevertheless it may be said, in a general way, that urobilinuria usually occurs at the height of the anemia (29), and most investigators believe that it has a relationship to an assumed increase in destruction of erythrocytes.

Albuminuria has been found in about 20 per cent of the cases and usually it has been of moderate degree.

The urine often gives a strongly positive test for indican; Schauman (381) found this test positive in every case in which he employed it. Eckert (109a) observed diminution of the reaction following expulsion of the worm; Grawitz (159a) stated that it became negative as soon as the improvement commenced; Schauman and Saltzman found in many instances the reaction for some time to be as strong after the worm had been expelled as it had been before. Becker (29) found a positive test for indican in eight of twelve cases in which the test was performed and in two of these the test was negative at a later time; he seemed to see a certain correlation between a positive test for indican and the presence of *Escherichia coli* in the stomach. Urobilinuria, albuminuria and indicanuria each is similarly present in cryptogenetic pernicious anemia (168).

In most cases, menstruation will cease at the beginning of the disease. Schauman (381) reported copious flowing in only one of his twenty-six cases. With recovery from the anemia the menstrual periods usually reappear. In established cases of cryptogenetic pernicious anemia, the menstrual cycle is nearly always discontinued, but it may return during remissions (168).

Of the symptoms referable to the central nervous system, tinnitus and dizziness are most common; in the far advanced cases fainting may occur when the patient tries to assume an upright position. A heavy feeling in the head, throbbing and severe headaches may occur. Sleep is often more or less disturbed. Psychic changes may take place. In disposition the patients may be irritable, sulky, cross, and difficult to please. Sometimes a certain clumsiness and slowness of speech is observed, possibly because the patient attempts to avoid the effort of talking. States of excitation with hallucinations, delirium (97, 343,

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354), and mania (12, 437) may develop in far advanced cases. The patient is usually seen to go into coma immediately before death; very few live after they have become comatose. States of neurosis and psychosis have been referred to as evidences of constitutional inferiority, when they occur in nonanemic carriers. The same basis for nervous disorders of course is present in those carriers in whom anemia develops as a consequence of the parasitism. The cases of Cramer and Ketcher already have been mentioned. Huber's (186) patient was definitely neurasthenic, the patient of Isaacs, Sturgis and Smith suffered from nervous irritability, irritable depression was present in Rubenstone's case, and dementia praecox in that of McGaffin.

Schauman and Saltzman referred to a case of Diphyllobothrium anemia in a sailor, aged thirty-five years, who denied syphilis or alcoholism, but who had had a stroke resulting in temporary loss of speech and paralysis of one arm and one leg. These phenomena lasted only a few hours. However, it would be reasonably safe to regard the sailor's statement with a certain amount of skepticism. Schauman and Saltzman also mentioned a case of involuntary jerking of muscles in the region supplied by the lower division of the facial nerve. This motor disturbance disappeared shortly after expulsion of the worm. In another case, according to anamnestic information, for three weeks there were strange tonic convulsions of the extremities.

Nervous and mental manifestations in wide variety are equally well known in cryptogenetic pernicious anemia (94) and the conditions described are therefore entirely consistent with this type of anemia.

In the spinal cord, changes may take place similar to those encountered in cryptogenetic pernicious anemia. Minnich reported two such cases from Königsberg, Germany, in which necropsy was performed and the clinical diagnosis of subacute combined sclerosis was verified. Both had paresthesia of the extremities and in both positive Romberg signs were elicited. Paresthesia, tingling and numbness are not uncommon complaints in cases of Diphyllobothrium anemia, but most of the writers do not seem to give this the significance generally ascribed to it. Minnich's cases are apparently the only two in which thorough neurologic examination has been undertaken. The fork vibration test is not referred to in only one instance (197). It is difficult, therefore, to get a true impression of the frequency of slight involvement of the spinal cord, and statistical data are not available by which this could be calculated. Evidently, gross complicating involvement of the cord is not as common in Diphyllobothrium anemia as in cryptogenetic pernicious anemia. On the other hand, the frequency of combined sclerosis and peripheral neuritis in cryptogenetic pernicious anemia varies. Woltman found it to be 80 per cent in a series of cases, whereas Türk had only two undoubted cases in his series of eighty cases and Schauman and Saltzman in a group of 520 cases (326 with Diphyllobothrium anemia and 194 with cryptogenetic pernicious anemia) saw definite involvement of the spinal cord in only three cases, is one of which, in addition, syphilis was present. Paresthesia of hands and feet was reported by Neubecker as occurring in his case, and by Saltzman (374) as occurring in three of a series of five cases. Numbness of the hands was present in the case of Isaacs, Sturgis and Smith, and increased, diminished or absent knee jerks in several of Rosenqvist's (354) cases, in one of which there was foot clonus, besides increased knee jerks. In Minnich's two cases the anemia and the combined sclerosis developed simultaneously. It seems impossible to comprehend the occurrence of these changes in the spinal cord without assuming the presence of partial constitutional inferiority, or special susceptibility on the part of the nervous system. It is startling indeed to note the extreme rarity of subacute combined sclerosis among Finlanders who seem to have, of all nationalities, the most marked susceptibility for pernicious anemia, of the cryptogenetic as well as of the Diphyllobothrium type.

Of all changes, those in the blood have attracted the greatest interest and have been studied most carefully.

No mention has been found in the literature of the total blood volume in Diphyllobothrium anemia. One gets an impression of diminished volume by observing the difficulty with which a drop of blood may be obtained from the finger tip, and postmortem examination supports this impression.

The specific gravity of the blood has been determined, by Hammerschlag's method, to be 1.032, 1.034, and 1.035, respectively, in three cases in which the hemoglobin content varied from 20 to 27 per cent; normal controls gave the average value of 1.060. Consequently, marked decrease in specific gravity evidently occurs.

The blood appears to be thin and coagulation takes place slowly (84, 381). Only once have the coagulation factors been determined, namely, in the case reported by Grünmandel, who found a bleeding time of eight minutes, a coagulation time of twenty minutes, and clot retraction time of more than two hours. The color of the blood is not always that of pale beef juice at the height of the disease. Often it has a dark amber, brownish, or even tarry appearance (341). If a drop of blood remains on the finger for a little while, small islands of a darker color may be discovered in a certain number of cases.

Rosenthal found a normal rate of sedimentation, and normal surface tension of the blood serum in his case; erythrocytes numbered 3,800,000 in each cubic millimeter of blood.

The blood serum has a characteristic dark amber to golden-yellowish color, due to its content of bilirubin and urobilinogen. Quantitative determinations have been done by Rosenthal, Lind (240), Isaacs, Sturgis and Smith, and Richter, Maurer and Eyl. Isaacs, Sturgis and Smith found a bile index of 60 and a concentration of serum bilirubin of 1.5 mgm. in each 100 cc. of serum, with indirect van den Bergh reaction. Richter, Maurer and Eyl found an index of 12.5 in their case; Rosenthal, an icteric index of 4.1 and a dilution of bilirubin of 1 to 35, and Lind, a Meulengracht reaction of 1 to 10 (indicative of slight bilirubinemia).

Isaacs, Sturgis and Smith found present an inhibitory effect of the serum on the growth of lupin seedlings in Shive's solution, as it is found in cryptogenetic pernicious anemia (256).

Quantitative chemical analysis of the blood has not been done systematically; the data refer to three cases studied by Sawjalow, Rosenthal, and Cameron and Foster. If their observations are compared with those of normal blood and of blood from patients with cryptogenetic pernicious anemia, only a few deviations from expected quantities are found. Thus Sawjalow met with a definite decrease of the phosphorus content both when determined in the serum ($P_20_5=0.019$ per cent) and in the ash ($P_20_5=2.60$ per cent). He also concluded that the erythrocytes contained about twelve times more fat (1.18 per cent) and about ten times less cholesterin (0.003 per cent) than was found in a case of cryptogenetic pernicious anemia. Cameron and Foster studied the erythrocytes, the hemoglobin, and the chlorides in blood from patients with cryptogenetic pernicious anemia as compared with normal controls. Included in their cases is one with "secondary anemia (from *Diphyllobothrium latum*)." I have calculated mean quantities in three groups of their subjects: ten normal persons, ten patients with cryptogenetic pernicious anemia (erythrocytes less than 2,000,000) and one case of Diphyllobothrium anemia. The cell volume in percentages was found to be somewhat higher than was anticipated (18.1 per cent), the cell chlorides in grams in each 100 cc. of centrifuged cells gave about twice the expected figure (0.207), and the cell hemoglobin in grams in each 100 cc. of centrifuged cells was only half of the expected quantity, namely 26, if Diphyllobothrium anemia was to be considered a "pernicious type." That is probably why the authors carefuly emphasized the secondary character of the anemia in their case, in spite of the fact that the color index was found to be 1.25. Rosenthal, in a case in which there was mild anemia (erythrocytes 3,500,000 and hemoglobin 52 per cent) found the concentration of blood sugar to be 141, and the carbon dioxide combining power of the blood plasma, 44, without sugar being present in the urine. These data are entirely too few to justify any definite conclusions.

The concentration of hemoglobin is regularly more or less diminished, in a degree varying from case to case and from one stage to another in the same patient. At the height of the disease it may sink as low as to 20 or 30 per cent of the normal. The lowest recorded figures percentage of hemoglobin are 10 (128, 272), 11 (203), and 15 (29, 114, 272) in patients who recovered, and 11 (354), 14 (29), and 15 (351) in patients who died. The low readings in Eckert's and Cramer's cases, 15 and 11 per cent respectively, occurred in a hypochromatic type of anemia with a fatal outcome. The distribution of the hemoglobin among the cells is remarkably uneven, in contrast to the rather equal distribution in normal individuals. Cells rich in hemoglobin are intermingled with those in which hemoglobin is of low concentration, and this irregularity, although following no strict rule, has a tendency to run parallel with the size of the erythrocytes in such a way that the larger cells contain relatively more and the smaller cells relatively less hemoglobin. This polychromatophilia has been recorded by Askanazy, Neubecker, Jawein, Bard, Herzog, Grünmandel, Grönberg (162), Edsall (114), Meyer (272), Lynah, Schmidt, Isaacs, Sturgis and Smith, Nicholson, Richter, Maurer and Eyl, and others.

The color index in Diphyllobothrium anemia as in cryptogenetic pernicious anemia and in contradistinction to what generally is called simple or secondary anemia, in most cases is 1 or greater than 1 (11, 14, 29, 32, 67, 79, 90, 97, 99, 109, 114, 162, 164, 170, 178, 196, 197, 203, 237, 238, 263, 284, 300, 302, 310, 328, 343, 347, 351, 380, 394, 410, 435, 450, 456, 478). The highest color index is reported by Neubecker and Orlowsky as 2.5 whereas Minnich found 2.33, and Cramer 2.2 in one each of their cases. Occasionally, however, this index is found to be slightly less than 1, at least at certain times in the course of the disease (14, 97, 99, 114, 128, 197, 203, 272, 354, 355, 376). A somewhat lowered color index, however, must be considered an exception from the rule (381, 389). Mention of the few instances in which hypochromatic anemia has been observed in connection with *Diphyllobothrium latum* has been made.

The color index, in practically all instances, is calculated directly. A modification upward would take place if the figures were corrected. By the methods most commonly employed, the reading for hemoglobin in normal persons with 5,000,000 erythrocytes in each cubic millimeter of blood runs from 75 to 90 per cent, whereas, in very few persons, it is as high as 100 per cent.

Studies of volume index apparently have not been done in Diphyllobothrium anemia.

The number of erythrocytes, as a rule, is definitely reduced and varies from case to case and from one stage to another in the same case. This reduction, as has been pointed out, is usually more marked than the decrease in concentration of hemoglobin. The numerical value of the erythrocytes usually has reached the level of about 2,500,000 before the patient consults a physician. Values less than 1,000,000, however, are not infrequently seen. Schauman and Saltzman have made up a table which shows graphically the erythrocyte count of the patients on admission (fig. 4). Compared with cases treated for cryptogenetic pernicious anemia the curves are definitely, characteristically different. This difference seems to indicate a more acute beginning in Diphyllobothrium anemia than in cryptogenetic pernicious anemia. Counts as low as 395,000 (381) 350,000 (354), 323,000 (211) and 390,000 (29) have been reported in fatal cases, and 621,000 (272), 468,000 (435), 608,000 (203), 575,000, and 410,000 (354) in patients who recovered, following expulsion of the worm; Orlowsky reported 300,000 in his case without stating the outcome. The upper limit for cases of Diphyllobothrium anemia necessarily will be arbitrary, as there are all intermediary steps between the levels found in cases of the disease and the normal level. Ragosa, in his series of carriers, found the number of erythrocytes to range from 5,600,000 to 2,670,000. In this study an erythrocyte count of 4,000,000 has been chosen as the line of separation between anemic and nonanemic carriers.

A great number of the erythrocytes present a variety of changes in form. Some assume the shape of pears; others that of clubs or anvils, or other weird shapes. This poikilocytosis is present to a greater or less degree and often, but not always, runs parallel with the severity of the anemia. Schauman (381) frequently observed less poikilocytosis in cases in which the count was below 1,000,000 than in cases in which it was between 1,000,000 and 2,000,000. It is also seen in cases of secondary anemia, although it is not as marked or as regular and is therefore of little diagnostic significance. A more important morphologic feature is the variation in size, anisocytosis, with the presence of large erythrocytes, macrocytes and megalocytes, first reported by Westphalen (471). These are considered by most investigators to be particularly characteristic of pernicious anemia. They are hardly ever absent, but probably are more frequently absent in Diphylloboth-

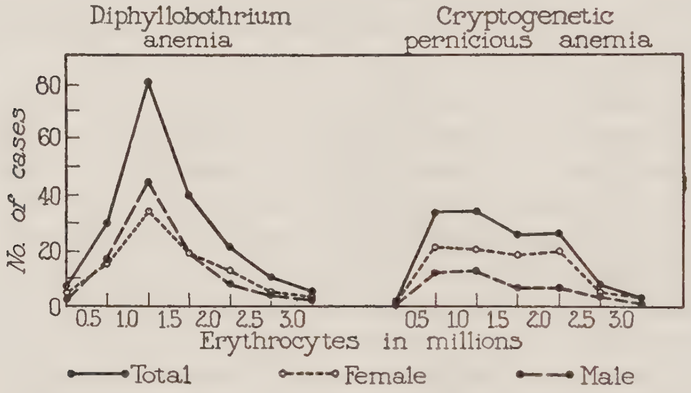

Fig. 4. Erythrocyte Count in Patients with Diphyllobothrium Anemia and Cryptogenetic Pernicious Anemia at Time of Admission to Hospital (Schauman and Saltzman)

rium anemia than in cryptogenetic pernicious anemia. Also, microcytes are found in great numbers, but measurements will disclose an

TABLE 12

Records at various stages of anemia (*Schauman* (381))

CASES	ERYTHROCYTES, MILLIONS											
	0–0.5	0.5–1.0	1.0–1.5	1.5–2.0	2.0–2.5	2.5–3.0	3.0–3.5	3.5–4.0	4.0–4.5	4.5–5.0	5.0–5.5	5.5–6.0
	Mean diameter, microns											
47		7.99		8.31				8.09		8.09		
48			7.65			7.95			7.77	7.84		
49			7.59		7.76		8.06		8.10			
50			8.22		8.39	8.49						7.90
51			7.74		7.86				8.07			
52				8.48	8.54	8.64	8.40		8.39		8.12	
53		8.05	8.50	9.14		8.27				8.22	7.89	
54			7.92	8.46				8.44		7.92		
55			7.88	8.04		8.14	8.11					
56				8.11					8.12			
57				7.88			8.25					7.89
58				7.81			7.74		7.52	7.75	7.75	
59			7.66		7.90			7.97			7.95	
60			7.57	8.09			7.81		7.95			
61			7.89			8.40		8.67				
62				7.90		8.22				8.01		
63		8.10						8.67	8.42			
64		8.16	7.91									
65			7.92				8.47		8.26			
66				8.01				8.29	8.32			
67			8.12			9.19		8.62				
68		7.82										
69	7.91											
70	7.89	7.84										
71			7.66		8.67			8.24				
72				7.75	8.31							
Compilation of 26 cases......	7.90	7.87	7.87	8.17	8.20	8.41	8.12	8.37	8.09	7.97	7.93	7.90

increase of the average diameter in the majority of the cases (114, 178, 197, 203, 240, 268, 272, 310, 317, 435, 478). Schauman (381), Schmidt and Neubecker, on the other hand, each reported one case in which the majority of the cells were normal in size or were of the microcytic type.

Schauman (381), in 1894, carefully measured the diameter of the erythrocytes in twenty-six of his cases, in most of them repeatedly during the course of the disease (table 12). The greatest diameter observed was 16 μ. He found that the number of small as well as of large types of cells was increased at the expense of those of medium size. His normal controls (five completely recovered cases of Diphyllobothrium anemia) gave the following percentage distribution: small cells, 1 per cent; medium-sized cells, 66 per cent, and large cells, 33 per cent. In the cases of Diphyllobothrium anemia there was constantly found to be an increase in number of small and large cells, with one exception. Schauman made the remark that the changes in size and form of the erythrocytes are not directly proportional to the degree of the anemia. In cases in which erythrocytes numbered less than 1,000,000, these changes have appeared less pronounced than in those with a count between 1,000,000 and 2,000,000. I have made tables and drawn some curves from data supplied by Schauman, and have found that the mean diameter of the erythrocytes is subject to considerable fluctuation. Figure 5, comprising Schauman's cases 47, 50, 52,

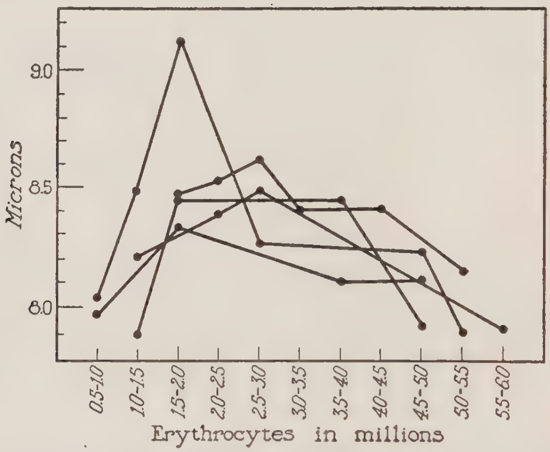

FIG. 5. FLUCTUATION OF MEAN DIAMETER OF ERYTHROCYTES IN FIVE SELECTED CASES OF DIPHYLLOBOTHRIUM ANEMIA (SCHAUMAN (381))

53 and 54, shows the diameter to be greatest, not at the height of the anemia, but at some point in convalescence, probably more often when erythrocytes number between 1,500,000 and 3,000,000. Figure 6, comprising cases 49, 51, 55, 59, 61 and 66, shows the mean diameter to be lower at the beginning and higher at the end of the hospitalization. Calculation of the mean diameter of the erythrocytes for all

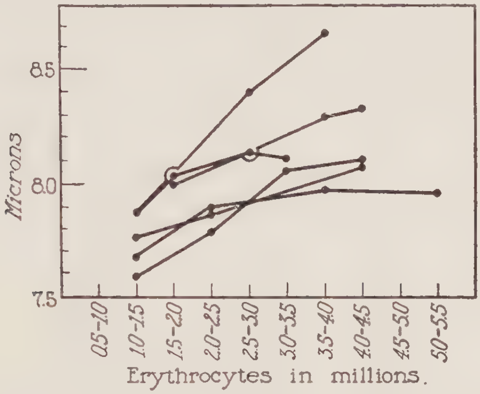

Fig. 6. Fluctuation of Mean Diameter of Erythrocytes in Six Selected Cases of Diphyllobothrium Anemia (Schauman (381))

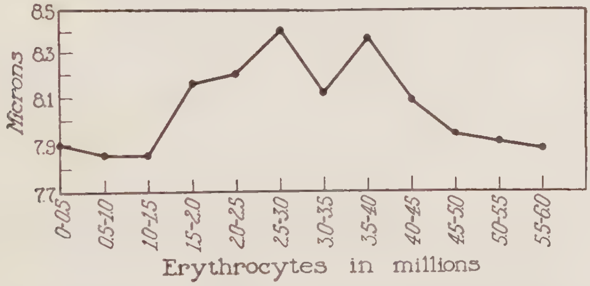

Fig. 7. Mean Diameter of Erythrocytes at Various Stages of Anemia; Calculated from Twenty-six Cases of Diphyllobothrium Anemia (Schauman (381))

of Schauman's twenty-six cases gives a combination curve as illustrated in figure 7. The striking feature of this curve is that the mean diameters are the same at the level of 500,000 as at the level of 6,000,000. The lowered average diameter of the cells in the extreme stage of the anemia may be a result either of marked increase in the number of microcytes or of marked decrease in the size of each microcyte. It may be explained by assuming that in this stage of anemia the reaction of the blood against the destructive influence is greatly increased over that evidenced in stages less advanced.

Careful search has not disclosed a similar study in cryptogenetic pernicious anemia; namely, on variation of the diameter of erythrocytes during the course of the disease. Isaacs, Sturgis and Smith constructed the Price-Jones curve for their case of Diphyllobothrium anemia, and demonstrated a remarkable likeness with cryptogenetic pernicious anemia; also they noted that the diameter, after treatment, went below normal (fig. 8). This depression may be only a temporary feature. I have constructed similar curves (fig. 9) for three of Schauman's cases, in two of which the curve conforms to that of Isaacs, Sturgis and Smith, and in one it is shifted to the left; that is, it has an average cell diameter smaller than normal.

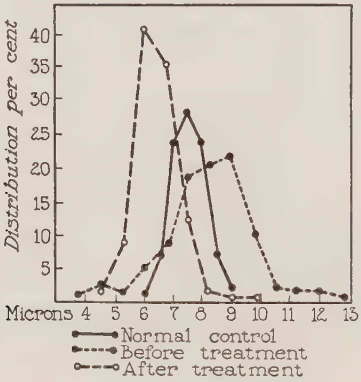

FIG. 8. "PRICE-JONES' CURVE" IN A CASE OF DIPHYLLOBOTHRIUM ANEMIA (ISAACS, STURGIS AND SMITH)

The microcytes are usually the cells which show the most marked changes in shape. The megalocytes commonly are round or oval and hyperchromatic, with a basophilic tinge.

Nucleated erythrocytes are seldom missing in Diphyllobothrium anemia when they are carefully sought. On the other hand, they are not very abundantly present except during blood crises, as seen in Jawein's case. Both normoblasts and megaloblasts may be found, the former always in greater numbers than the latter. Schauman and Saltzman defined a megaloblast as a nucleated erythrocyte with immature, finely granulated or reticulated nucleus, often, but not necessarily containing one or more nucleoli, and, as a rule, with a more or less strongly basophilic cytoplasm. Large erythroblasts, with pyknotic nuclei, and coarse, clumpy chromatin, are considered by them to be macroblasts (normoblasts); in the latter type, the nucleus often will have the shape of a rosette or clover leaf, and sometimes more or less complete fragmentation takes place. In smears may be seen free, extracellular pyknotic nuclear fragments, possibly, in most instances, artefacts. Once in a while, mitotic figures are observed. Various other phenomena have been observed, like basophilic stippling, Howell-Jolly bodies and Cabot's ring bodies. Basophilic stippling is more frequently seen in the smaller cells. The earlier observers, Reyher,

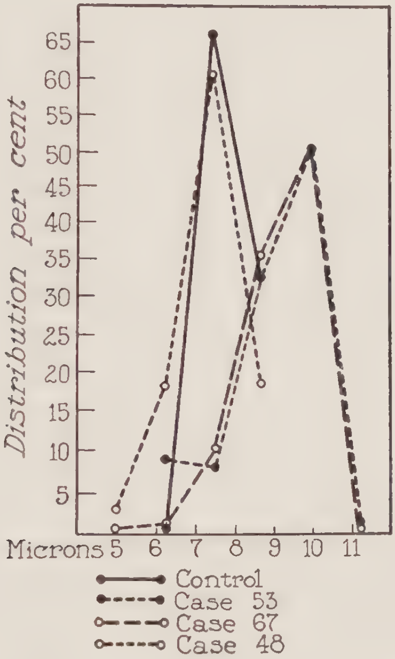

FIG. 9. ATTEMPTED CONSTRUCTION OF "PRICE-JONES' CURVES" IN THREE CASES OF DIPHYLLOBOTHRIUM ANEMIA (SCHAUMAN (381))

Schauman (381), Rosenqvist (354), and others, noticed the lack of rouleaux of erythrocytes in Diphyllobothrium anemia, and this lack was particularly marked in advanced stages of the disease. This feature is seen only in pernicious anemia to the same degree, although it is not foreign to secondary types.

The fragility test has been employed in a few instances. Herzog obtained hemolysis in from 0.38 to 0.24 per cent of sodium chloride solution. Increased resistance was present in the case reported by Courmont and André. Appelberg (8), in Schauman's clinic, compared the resistance in nine cases of Diphyllobothrium anemia and in eight cases of cryptogenetic pernicious anemia, with a series of normal controls. His observations may be summarized as follows: The minimal resistance was about normal in twelve cases, definitely lowered in four, and somewhat increased in one case. The maximal resistance was more or less increased in more than two-thirds of the cases. The width of resistance was generally increased; normal maximal resistance usually was associated with definitely lowered minimal resistance. The departure from normal was, at an average, greater in cryptogenetic pernicious anemia than in Diphyllobothrium anemia. Generally speaking, the width of resistance was greater in advanced cases than in lighter cases. No definite relation, however, could be established between the anemic condition on one side and the resistance of the erythrocytes on the other.

The average leukocyte count is generally somewhat lowered in Diphyllobothrium anemia, more so in advanced cases than in incipient cases, or when the patient is recovering. There are, however, great individual variations in this respect. In Schmidt's case with an anemic condition exemplified by erythrocytes numbering 810,000 and a concentration of hemoglobin of 20 per cent, leukocytes numbered 7500. Becker (29), on the other hand, reported 4600 leukocytes in a patient who had just recovered from an attack of anemia, and whose erythrocytes numbered 4,400,000.

Vierioujski observed the lowest number of leukocytes on record; namely, 704 in each cubic millimeter in a case in which erythrocytes numbered 744,000 and the concentration of hemoglobin was 22 per cent. The highest count was reported by Riley (348), 16,200, in a case in which erythrocytes numbered 3,422,000 and the concentration of hemoglobin was 65 per cent. Mustelin (295) found in his cases a minimal value for leukocytes of 2360 in a case in which the concentration of hemoglobin was 68 per cent and a maximal value of 8500 in a case in which the concentration of hemoglobin was 83 per cent before expulsion of the worm. Schauman and Saltzman tabulated forty-six average leukocyte counts in Diphyllobothrium anemia, correlated with the degree of anemia, and compared them with parallel counts in cases of cryptogenetic pernicious anemia, and they demonstrated moderate leukopenia in both. However, the reduction in number of leukocytes is not quite as marked in Diphyllobothrium anemia as in cryptogenetic pernicious anemia (table 13). At the same time, they called attention to a source of error in the technic of this count. According to Schauman and Saltzman, usually, when standard methods of counting are used, other nucleated cells are not distinguished from leukocytes; even erythroblasts are included in the total. This may account for some of the rather high leukocyte counts seen in crises when the blood is known to be flooded with nucleated erythrocytes. This source of error is illustrated in table 14. Mustelin (295) stated that the diagnostic sig-

TABLE 13

Leukocyte count at different stages of anemia (Schauman and Saltzman)

HEMOGLOBIN	CRYPTOGENETIC PERNICIOUS ANEMIA		DIPHYLLOBOTHRIUM ANEMIA					
			Before expulsion of worm		After expulsion of worm		Total	
	Number of counts	Average number of leukocytes in each cubic millimeter of blood	Number of counts	Average number of leukocytes in each cubic millimeter of blood	Number of counts	Average number of leukocytes in each cubic millimeter of blood	Number of counts	Average number of leukocytes in each cubic millimeter of blood
per cent								
20	9	3,741	2	3,350			2	3,350
20–29	25	3,726	3	4,121			3	4,120
30–39	39	3,410	6	4,787			6	4,787
40–49	19	3,672	2	4,435			2	4,435
50–59	12	3,427	5	4,738	3	5,933	8	5,186
60–69	3	2,147	1	2,360	7	5,890	8	5,249
70–79	2	3,230	3	3,133	5	6,048	8	4,955
80 and more	1	3,660			9	7,012	9	7,012

nificance of leukopenia is of less importance than previously has been assumed. The reduction in total number of leukocytes usually is not evenly distributed among the different types. Usually, the number of polymorphonuclear leukocytes is reduced proportionately more than the number of lymphocytes; thus, relative lymphocytosis is the result.

TABLE 14

Technical error in the counting of leukocytes (Schauman and Saltzman)

NUCLEATED CELLS IN EACH CUBIC MILLIMETER OF BLOOD	ESTIMATED LEUKOCYTES	ESTIMATED ERYTHROBLASTS
7,300	5,800	1,500
10,800	8,780	2,020
15,600	7,950	7,650
11,920	3,560	8,360
6,800	2,820	3,980

TABLE 15

Variability of the proportion of polymorphonuclear leukocytes to lymphocytes in Diphyllobothrium anemia

AUTHOR	HEMOGLOBIN	ERYTHROCYTES	LEUKOCYTES	POLYMORPHONUCLEAR LEUKOCYTES	LYMPHOCYTES
	per cent	*millions*		*per cent*	*per cent*
Richter, Maurer and Eyl	25	1.080	2,200	16.0	64.0
Cohen	15	0.700	5,000	34.0	58.0
Meyer (272)	15	0.780	5,000	37.0	54.0
Winiarski	22	0.723	14,600	39.1	60.9
Thompson	20	0.608	2,000	70.1	26.3
Grönberg (162)	40–50	1.520	8,000	72.0	26.0
Rosenthal	52	3.500	5,400	74.0	22.0

Great variations are observed, however, both from case to case, and in the same case from time to time. There is a return to polymorphonuclear preponderance with recovery. Table 15 illustrates the great variability of the proportion of polymorphonuclear leukocytes to lymphocytes. In regard to absolute numbers, only few data have been gathered (381, 424), too few to allow conclusions to be drawn from them. Also the lymphocytes must be reduced numerically in those cases in which the total leukocyte count goes down to, or below 1200 to 2000, the normal figure for lymphocytes in each cubic millimeter. Immature cells, myelocytes and myeloblasts, are, at least temporarily, present in a certain number of cases (164). Mustelin (295) found myeloblasts in about 50 per cent of the cases, but only at the height of the disease and in small numbers. Only in exceptional instances did they amount to 1 per cent of the number of leukocytes. They were all what might be called normal myeloblasts and were not of the pathologic types seen in leukemia. The appearance of these immature cells seems to indicate a deep-seated functional disturbance of the myelogenous centers. Qualitative changes may be seen in the neutrophilic leukocytes, expressed in a shift to the right of Arneth's formula, equivalent to an increase in number of aged cells. With increased segmentation of the nucleus usually goes an increase in the size of the cell. Young, immature forms may, however, also be seen.

Eosinophilic leukocytes usually are present in subnormal number. Meyer (272), Lynah, Cramer, Rosenow (351), Wallace and Grant, and Haden reported absence of these cells. Occasionally, however, eosinophilia is seen even in cases of profound anemia. Bard reported 7.5 per cent of eosinophils in a case in which erythrocytes numbered 1,700,000, and the concentration of hemoglobin was 25 per cent; Grünmandel reported 8 per cent in a case in which erythrocytes numbered 880,000 and the concentration of hemoglobin was 18 per cent, and Riley (348) reported eosinophils to be 14 per cent in a case in which erythrocytes numbered 3,420,000 and the concentration of hemoglobin was 65 per cent. Nicholson found a high eosinophil count in one of his cases in which erythrocytes numbered 2,500,000 and the concentration of hemoglobin was 65 per cent, but he did not state the percentage.

In regard to large mononuclear and transitional cells Ragosa demonstrated a decrease in their number, which ran parallel with the severity of the anemia, and in far advanced cases they might even be missing completely. As exceptions to this general rule may be cited Richter, Maurer and Eyl's case, in which there were 20 per cent monocytes and 920,000 erythrocytes, and Bard's case, in which the proportion of monocytes was 11 per cent and the number of erythrocytes 1,900,000.

A characteristic feature is the low platelet count, thrombopenia. Grünmandel reported 25,000, Cohen 30,000, Nicholson 45,000, Schauman (381), 68,000 and 75,000, and Herzog 90,000, whereas Rosenthal gave the highest count, 200,000, in a case of mild anemia in which erythrocytes numbered 3,500,000 and the concentration of hemoglobin was 52 per cent.

The blood picture during remissions and recovery will be considered later.

At this point it may be opportune to mention a few cases in which aplastic anemia has been diagnosed and ova of *Diphyllobothrium latum* have been found in the intestine (219, 221, 302, 369). The relationship of aplastic anemia to pernicious anemia has been under consideration for years and is not wholly settled as yet. The majority of cases of aplastic anemia present a picture different from that of pernicious anemia; the outstanding characteristics (76, 147, 406) are as follows: (1) two-thirds of the cases are in women; (2) 75 per cent of the cases are in persons aged less than thirty-five years; (3) the course is rapid and progressive without remissions; (4) marked hemorrhagic diathesis is present, notable in two-thirds of the cases reviewed; (5) the color index is 1 or less than 1; (6) abnormalities of size and shape of erythrocytes are absent; (7) hemolysis is not demonstrable; (8) glossitis is absent; (9) achlorhydria is absent; (10) lesions of the central nervous system are absent; (11) the bone marrow is aplastic; (12) regenerative signs, such as the presence of erythroblasts, reticulocytes, stippled cells and polychromatic cells are not seen; (13) there is intense leukopenia; (14) the diminution of granulocytes is particularly marked effecting relative lymphocytosis, and (15) marked thrombopenia is present.

Schauman and Saltzman pointed out, however, that in some instances of profound anemia in which the course is rapid and progressive and the anemia is non-regenerative in type, with dominating hemorrhagic diathesis and which probably should be classified as cases of aplastic anemia, several of the more important symptoms characteristic of pernicious anemia have been seen; for instance, megalocytosis, high color index, urobilinuria, achylia and sore tongue. In these cases, an approach toward pernicious anemia can hardly be denied. They also emphasized that in a certain number of cases of typical pernicious anemia a blood picture suggestive of aplastic anemia may be encountered in certain phases of the disease, and perhaps more frequently in its final stage, indicating a state of extreme exhaustion of the hematopoietic system. It does not always seem practicable to draw too distinct a line between the two types of anemia.

Unfortunately only one detailed report of a case of aplastic anemia associated with *Diphyllobothrium latum* is available in the literature, (219, 369). Kriloff's article is written in Russian and has not been obtainable. Nicholson's report is abbreviated.

Krantz adhered to the contention that aplastic anemia has some relationship to pernicious anemia in the sense that the bone marrow in a case of pernicious anemia may become exhausted from the heavy and continued drain and may change from a state of hyperplasia to one of hypoplasia and aplasia. His patient was a man, aged nineteen years, with a negative history except for infestation with a tapeworm of several years' duration. Frequent attacks of epistaxis developed, then bleeding from the gums, and the patient came under observation in a pale, emaciated condition. He had petechiae over the lower extremities and retinal hemorrhages. He had some fever, felt dizzy, and complained of headaches. The heart was enlarged, and a systolic murmur could be heard. The test for indican in the urine was slightly positive. The first examination of blood revealed 860,000 erythrocytes, a color index of 1.16, poikilocytosis, slight polychromatophilia, some anisocytosis, with megalocytes predominating, very few normoblasts, 3000 leukocytes, 28 per cent polymorphonuclear neutrophilic cells, 6 per cent transitional cells, 66 per cent lymphocytes (almost exclusively small ones), and no eosinophils. No information was given as to the reticulocytic count, the platelet count or the coagulation factors. The course was rapid and progressive and the patient died three and a half months after onset. The last blood count, on the day of death, revealed 332,000 erythrocytes, hemoglobin below 10 per cent, color index 1.5, 800 leukocytes, 96 per cent lymphocytes, 4 per cent polymorphonuclear neutrophilic cells, and one megaloblast. At necropsy slight hemosiderosis of the spleen and marked hemosiderosis of the liver were found. The bone marrow of the femur and ribs was aplastic, fatty, and contained stippled and markedly polychromatic erythrocytes. These cell types had not been found in the peripheral blood. In the intestines were found several pieces of *Diphyllobothrium latum.*

It seems fairly certain that this was a case of aplastic anemia. If a relationship between the aplastic and the pernicious type exists, then it is possible to imagine that the fish tapeworm, in this instance, may have been of etiologic significance. On the other hand, no insistent objection can be raised against Frank's contention that the case represents only coincidence.

The account of Tallqvist's (433) patient who at one time suffered from typical Diphyllobothrium anemia, and after a free interval, succumbed to aplastic anemia, lends support to the contention of Krantz regarding the relationship of aplastic anemia to pernicious anemia.

Chronic myelogenous leukemia was the clinical and hematologic diagnosis in the case reported by Stukowski. Incidentally segments of Diphyllobothrium were discovered in the stool, and, by means of a vermifuge, one *Diphyllobothrium latum* and one *Taenia saginata* were recovered. The outcome of the case was not mentioned.

Course. Untreated Diphyllobothrium anemia seems to have a greater progressive tendency than the cryptogenetic variety. The duration also seems to be shorter as a rule, and remissions are usually a less constant feature. If the attack is characterized by a rapidly progressive course, the patient as a rule will come under observation during that attack. If, on the other hand, the condition develops slowly, the history often will be one of an intermittent course, periods of disease alternating with periods of well-being and the duration of the more or less free intervals varying greatly, from weeks to years. Rosenqvist (354), was the first to observe that exacerbations tended to occur in the spring. In two of his cases there were several exacerbations, three and ten respectively, all of which occurred in the spring of consecutive years. Schauman (381, 383), Shimanskiy, Barron, and others also recorded cases with remissions. It has been seen that Diphyllobothrium anemia may lead to death within two months after the appearance of the first symptoms; also, that it may extend over several months or years. Therefore, the course may be designated acute, subacute or chronic, accordingly.

The beginning of a remission is often very sudden; the first sign usually is a subjective feeling of well-being. Clinically there is gain in strength and color, quieting of gastro-intestinal disturbances, and subsidence of whatever other associated symptoms the patient may have been complaining of. Hematologically, beginning recovery is characterized by a reticulocytic reaction, a transitory increase in the number of nucleated erythrocytes, progressive increase in the number of erythrocytes and in the content of hemoglobin with a slow and gradual lowering of the color index to normal. At times the blood is practically flooded with nucleated erythrocytes, and creates a situation which has been named a "blood crisis" (203). The leukocyte count tends to approach normal, and in certain instances so marked an increase is observed that the condition is one of real leukocytosis (354, 389). The error of including nucleated erythrocytes in this count has been pointed out. Mustelin (295) did not observe leukocytosis.

TABLE 16

Influence of age on regeneration of erythrocytes in patients recovering from Diphyllobothrium anemia (Schauman and Saltzman)

AGE	CASES	TREATMENT	INCREASE IN ERYTHROCYTES AFTER TREATMENT	MEAN DAILY INCREASE OF ERYTHROCYTES FOR EACH CUBIC MILLIMETER
years		*days*	*millions*	*thousands*
0–19	21	40.3	2.204	54.7
20–29	32	46.7	2.463	52.7
30–39	19	43.9	2.110	48.0
40–49	17	48.8	2.394	49.1
50–59	13	44.9	2.038	45.4
60 and more	5	50.7	1.636	32.3

As the patient recovers, the intense signs of regeneration disappear, but megalocytosis may persist for some time. Gradually the constituents of the blood return to normal, and when full health is attained, little if any evidence remains of the former serious state of affairs. The rapidity with which regeneration of the blood takes place varies with age. Table 16 shows this distinctly; a noticeable decline in regenerative activity is observed with increasing age.

There is only vague indication of a greater recuperative power with greater initial intensity of the anemia, not enough on which to base definite conclusions.

Urobilinuria, which is present during the decline of the condition, may become accentuated at the beginning of a remission, but regularly it disappears during improvement.

In instances in which the outcome is fatal, the patient becomes physically exhausted and mentally inert and apathetic; he goes into a soporific state, which may develop into coma. Occasionally patients are reported as being irrational toward the end (97, 203, 407).

Prognosis. Before the significance of the fish tapeworm and the beneficial effect of successful treatment for expulsion of the worm had been learned, the prognosis in Diphyllobothrium anemia was practically as unfavorable as that in cryptogenetic pernicious anemia. One even gains the impression that the course in the former was more fulminant. Since the establishment of rational anthelmintic treatment as a standard requirement in all cases of Diphyllobothrium anemia, the prognosis has been improved in a most remarkable manner.

Schauman (381) found in his early series a mortality of 16.7 per cent. Of the twelve patients who died three could not be given the proper anthelmintic treatment on account of their moribund condition, and one died an hour after administration of the vermifuge. If these four cases are excluded the rate of mortality is reduced to 11.8 per cent. It was also found that the prognosis depended largely on the degree of severity of the anemia at the time of observation. Schauman summarized his analysis as follows: The prognosis is good when the erythrocytes number more than 1,000,000 in each cubic millimeter of blood; very poor when it goes below 1,000,000; not entirely hopeless, even if the count drops to about 500,000 and less favorable in the presence of a complicating organic disease.

As time has passed and knowledge of the condition has spread in Finland, the mortality there has steadily decreased, due to early diagnosis, and to rational application of adequate treatment. Otherwise, the prognostic points which Schauman made in 1894 stand unchanged, if the effect of the recently instituted treatment with liver is left out of consideration.

The rate of mortality is not influenced by sex. Age, however, appears to have some bearing on the prognosis (table 17); the mortality rate increases markedly with age. Comparison with cryptogenetic pernicious anemia, in this regard, reveals that in the latter condition the mortality is only slightly different in the three main age groups;

the greatest mortality is in the age group 0 to 19 years. This compilation, however, takes only hospital cases into consideration, and as it is well known that many patients leave the hospital during a remission, later to die in their homes or in some other hospital, the mortality rate of the cases of Diphyllobothrium anemia becomes rather strikingly favorable in comparison.

Patients cured of Diphyllobothrium anemia as a rule have a shorter expectancy of life than the average person of the same age. One cannot definitely say whether this is due to remote effects of the anemia or whether it is an expression of inherent constitutional inferiority. Curiously, many such persons succumb to tuberculosis later in life.

TABLE 17

Age distribution of mortality in hospital before introduction of treatment with liver (Schauman and Saltzman)

AGE	DIPHYLLOBOTHRIUM ANEMIA				CRYPTOGENETIC PERNICIOUS ANEMIA			
	Cases	Deaths	Mortality		Cases	Deaths	Mortality	
years			*per cent*	*per cent*			*per cent*	*per cent*
0–19	52	4	7.7	6.8	6	5	83.3	44.1
20–29	94	6	6.4		28	10	35.7	
30–39	74	9	12.2	14.4	45	24	53.4	45.4
40–49	67	11	16.4		49	17	34.7	
50–59	54	8	14.8	31.0	47	23	48.9	52.5
60 and more	29	9	31.0		40	21	52.5	
Total........	370	47	12.7		215	100	46.5	

The average duration of hospitalization of sixty patients who recovered was forty-eight and five-tenths days. Some of these were cured, and others were convalescing, on dismissal. In a given case in which the prognosis is pronounced good, in which there are no complications, and in which treatment has been directed against the cause, nearly complete restitution should be effected in six to eight weeks.

Schauman (383) in 1910 published a report of reinvestigation of the seventy-two cases he had used as material for his monograph in 1894. Twelve patients had died while in the hospital, three were untraceable, and fifty-seven were left for analysis. Of these fifty-seven patients, fourteen had died in the interim, six of pulmonary tuberculosis (almost 50 per cent) and one each of the following diseases: carcinoma of the stomach, carcinoma of the uterus, Laennec's cirrhosis of the liver, myocarditis, valvular cardiac lesion, arterosclerosis with psychosis, typhoid fever, and accident. Not one patient had died of recurrence of anemia. Death occurred from one and a half to twenty-four years after dismissal from the hospital following treatment for Diphyllobothrium anemia. Forty-three patients were still living, five of them twenty-five or more years after dismissal, and thirty-five of them seventeen to twenty-four years after dismissal. Sixteen of these were in excellent or in good health, whereas twenty-seven had been or were sick. Of these twenty-seven, the nature of the illness was recorded in twenty-one; in three, it was arteriosclerosis; in three, chronic disease of the lungs (apparently tuberculosis); in four, alcoholism; in one, epilepsy; in two, mental disease; in seven, recurrent attacks of anemia, and one patient was characterized as a "vagabond."

Schauman and Levander, seven years later, reported on the condition of the blood in twenty-one cases which were traced, fifteen of which apparently were not included in Schauman's paper of 1910. Table 18 presents the blood counts in these thirteen cases, together with eight of Schauman's earlier cases. In only one instance was the erythrocyte count below 4,500,000 (namely, 4,000,000), and the concentration of hemoglobin below 80 per cent (namely 73 per cent), and in only three cases was the leukocyte count lower than 5000 (namely 4700, 4600 and 4150 respectively). Montandon found his patient in excellent health eleven and a half years after successful anthelmintic treatment for Diphyllobothrium anemia.

Treatment. A formidable weapon exists in dealing with Diphyllobothrium anemia. Even before this type of anemia had been clearly defined the beneficial effects of successful anthelmintic treatment had been noted (55, 56, 181, 341, 363, 366); the prognosis, which until that time had been almost unfailingly fatal, changed practically over night. In short sequence it became a routine procedure to examine, for ova of *Diphyllobothrium latum*, the feces of all patients suffering from pernicious anemia and to administer a vermifuge with the least possible delay on finding ova. The fact that some patients might go into a remission on an indifferent regimen, and without expulsion of the worm, did not justify a passive attitude. Likewise, even patients whose condition must be characterized as extremely serious were offered this chance for recovery, although anthelmintic treatment is admittedly an exhausting procedure. It should be kept in mind that ova are sometimes difficult to find. Even repeated examination may fail to reveal their presence; in a few instances they were detected only after the patient had already recovered from the anemia. Several clinicians, therefore, would administer an anthelmintic even in cases in which examination of stools had resulted negatively.

TABLE 18

Blood counts obtained on reëxamination in twenty-two cases of diphyllobothrium anemia (Schauman and Levander)

YEARS ELAPSED SINCE ATTACK OF ANEMIA	HEMOGLOBIN	ERYTHROCYTES	LEUKOCYTES
	per cent	*millions*	
24*	93	4.93	4,700
24*	96	5.20	7,400
23*	98	4.90	5,500
23*	85	4.60	7,400
21*	80	4.87	8,000
21*	90	4.70	6,600
21*	85	4.63	5,000
21*	89	4.80	9,300
18	95	5.20	7,200
18	94	4.78	7,000
16	86	4.60	7,000
11	85	4.00	6,400
9	90	5.10	8,000
9	82	4.70	8,000
9	104	5.77	4,600
8	82	4.75	7,540
7	85	5.00	5,600
7	92	5.56	8,000
7	90	4.80	7,200
3	73	4.53	4,150
2	87	5.11	6,700

* Cases previously reported by Schauman (381).

Of recent years, a diet containing an ample proportion of liver has been found markedly to stimulate the hematopoietic organs and rapidly to incite remission in cryptogenetic pernicious anemia (286). It seemed reasonable to try this dietary measure also in Diphyllobothrium anemia since it appeared to be in all essentials identical with the cryptogenetic type. Benefit from treatment with liver in anemia from infestation with tapeworm, without elimination of the worm, has been seen by Schottmüller, Warthin, (464), Becker (22), and others, and a typical response of reticulocytes, such as that observed in cryptogenetic pernicious anemia, has been recorded by Kerr and Falconer Isaacs, Sturgis and Smith, Richter, Maurer and Eyl, Vesa, and Saltzman (373, 374). That a similar reticulocyte reaction may follow expulsion of the parasite, without administration of liver, has been shown by Belonogova and Saltzman (374), in this instance, however, the response seems to be somewhat slower in developing. In a case in which the condition had improved until the concentration of hemoglobin was 88 per cent and erythrocytes numbered 3,400,000 following administration of a vermifuge, acceleration and completion of the recovery followed the administration of liver (241). Atypical reactions are occasionally seen. Saltzman (374) recorded a case which was refractory to treatment with liver, but which later responded typically after a vermifuge had been given. It seems as though Diphyllobothrium anemia, more often than cryptogenetic pernicious anemia, was resistant to treatment with liver. Saltzman (374) suggested as a working hypothesis, that the liver may focus more on the endogenous factor, or on the presupposed insufficiency of organs, than on such exogenous influences as, for instance, that represented by *Diphyllobothrium latum.*

Isaacs, Sturgis and Smith noted eosinophilia after they had started feeding their patient liver (liver response). The same response is observed in cryptogenetic pernicious anemia. Treatment with stomach tissue or its extract has not been reported as yet. There is no reason to believe this treatment less effective in Diphyllobothrium anemia than in cryptogenetic pernicious anemia.

The best treatment in a case of Diphyllobothrium anemia, at the present time, would seem to be a combination of anthelmintic measures and administration of liver. The liver should be given before the anthelmintic when the general condition appears too serious for safe administration of a vermifuge, and sufficient time should be allowed for restitution of strength and resistance. A high-calorie, high-vitamine diet should be given with half a pound of liver, or its equivalent in extract, daily and the effect on the blood picture should be carefully watched. When the patient is ready for the more drastic part of the treatment, a suitable anthelmintic should be administered, following a technic that has been proved to be safe and effective.

The standardized method used in The Mayo Clinic (262) is as follows: On the day preceding the administration of the anthelmintic the patient may have breakfast; for lunch and supper he may take only black coffee; tea without cream, and water, may be substituted. At 6 p.m., the patient is given 15 to 30 grams of magnesium sulphate and this dose is repeated the next day at 6 a.m. That morning breakfast is not given. After the bowels have moved 30 cc. of the following emulsion is administered: oleoresin of aspidium 6 grams; powdered acacia 8 grams, and distilled water to 60 cc. One hour afterward the other 30 cc. of the emulsion is given, and this is followed two hours later by 30 grams of magnesium sulphate. After another two hours have elapsed a large soapsuds enema is given.

The stool is received in a container, strained through a 20-mesh sieve, and flushed with warm tap water. No toilet paper is allowed. The contents of the sieve are emptied into a flat enamel pan measuring about 25 by 30 cm., the bottom of which has been painted black with asphalt paint. The sieve is rinsed by running water through the mesh, bottom up, into the pan.

A careful search for the head of the worm should be carried out, and if it is not found the stool should be examined for ova at intervals. If ova persist, then the treatment for expulsion of the worm should be repeated.

Experience regarding the course and the prognosis of Diphyllobothrium anemia has shown that most patients remain cured after the parasite has been removed. It is questionable, therefore, whether continued administration of liver is as necessary as it has been found to be in cryptogenetic pernicious anemia. After complete restitution, the physician's attitude should be that of watchful waiting, interspersed with periodic reëxamination so that a set-back can be treated in its initial stage.

Lindström (243) found blood transfusion to be of great palliative benefit when the condition seemed critical.

Aside from this causal and active treatment, such general measures as good food, fresh air, sunlight, moderate exercise, and avoidance of excessive physical and mental stress should not be neglected. Especially during the winter months, a sufficient supply of vitamines should be provided.

Pathology. The extreme pallor is striking; often a slightly yellowish tinge is present. The internal organs also are profoundly anemic, and hypostatic congestion, to any considerable degree, is lacking.

Subserosal and mucosal hemorrhages in the form of petechiae are observed in various parts of the body, and small, scattered hemorrhages are also seen in the meninges of the brain and the spinal cord. Disseminated parenchymatous bleeding also may be found. Biruli reported a case in which at necropsy numerous hemorrhagic areas were found in the pia-arachnoid of the cerebellum and occipital lobes, and also in the cerebellum and corpora striata.

TABLE 19

Iron content of liver and spleen in average percentages (by weight) of the dried substance

CONDITION	AUTHOR	CASES	LIVER	SPLEEN
Normal	Hunter (190)	4	0.080	0.231
	Schauman (381)	4	0.080	
	Appelberg (9)	2	0.172	0.112
Pernicious anemia	Hunter (190)	12	0.325	0.175
	Schauman (381)	2	0.185	
	Appelberg (9)	7	0.395	1.049
Diphyllobothrium anemia	Hunter (190)			
	Stockman	2	0.214	0.366
	Schauman (381)	2	0.211	
	Appelberg (9)	3	0.690	1.013

Fatty changes of the various organs, especially the heart and liver, are common.

Hemosiderosis of the liver, spleen, lymph nodes and bone marrow may be considerable, and is almost always present. There is no significant difference in content of iron between cryptogenetic pernicious anemia and Diphyllobothrium anemia (table 19).

The marrow of the long bones is, to a greater or less degree, changed in color from fatty yellow to red, such as that of raspberry jelly. Similar changes are seen in the short bones. Macroblasts and megaloblasts, macrocytes and megalocytes, and usually also normoblasts and normocytes are found more or less abundantly. These megaloblastic changes may not be uniformly present, but may be seen in spotted distribution; other regions may have a normal appearance. Leukocytes usually are shifted to the immature side according to the Arneth formula; sometimes the granular types, sometimes the lymphoid types predominate. The bone marrow presents not only a complicated but a greatly varying picture, for the anemia itself is shifting.

Myelogenous centers, erythroblastic, myeloleukoblastic, or combined, may appear outside the bone marrow, principally in the spleen.

The liver and the spleen vary somewhat in size, but enlargement does not belong to the typical picture of Diphyllobothrium anemia.

Nothing remarkable is found in the kidneys aside from fatty changes and a slight amount of hemosiderosis.

Roos found great reduction in lipoids and in chromophile substance of the suprarenal glands and also, other changes of varying nature and uncertain value, equally present in cryptogenetic pernicious and Diphyllobothrium anemia.

The interest in the pathologic changes of pernicious anemia early centered in the gastro-intestinal tract. Rapidly developing postmortem changes have confused the picture. Möller found chronic catarrhal and atrophic changes of the gastric and intestinal mucosa in ten cases of Diphyllobothrium anemia, and the same in three cases of cryptogenetic pernicious anemia; it was also present in the case of Eckert (109). The degree of the changes apparently depended on the duration of the anemia; the pathologic changes were quantitatively slightly less in Diphyllobothrium anemia, which as a rule has a shorter and more fulminant course. Wallgren (457, 458) undertook a pathologic study of a series of fourteen cases of cryptogenetic pernicious anemia and of two of Diphyllobothrium anemia. The two types did not differ from each other. The mucosa of the tongue, esophagus and stomach showed chronic inflammatory changes in every case; the mucosa of the small intestine showed the changes in at least 75 per cent, and of the large intestine in at least 40 per cent of the cases. These changes were characterized by degeneration of the epithelium and round-cell infiltration of the deeper layers. The papillae filiformes and fungiformes of the tongue were poorly developed, often almost completely lacking. Both the primary and secondary papillae of the submucosal connective tissue were shorter, plumper, and wider. Similar changes were also demonstrated in the esophagus. The epithelium of the tongue and esophagus was often lower than normal, and, on the tongue, it was not infrequently faultily cornified. The gastric mucosa was usually thinner than normal. In the intestine, the changes were much less pronounced, and real atrophy could not be demonstrated. It was interesting to note that apparently there was generalized increase of the amount of connective tissue in the gastro-intestinal wall. This connective tissue seemed denser and of finer fibrillary structure than that normally seen. Schauman and Saltzman expressed the opinion that this has a certain significance; that it is not so much a sequela of a chronic inflammatory process as an expression of cirrhosis, a tendency to form more connective tissue than normal. Similar cirrhotic processes were found in the kidneys and liver (389). In cryptogenetic pernicious anemia, Ziegler, Roth, Paszkiewicz, and others have found the same increase of connective tissue. Several circumstances seem to indicate that the pathologic changes in the gastro-intestinal tract may be explained, at least partly, on the basis of inferiority of the mucosa.

In the myocardium, as a rule, there is a marked degree of fatty degeneration.

Pathologic changes in the central nervous system have been referred to by Lichtheim and Minnich, and in their cases the features characteristic of subacute combined sclerosis, as it is seen in cryptogenetic pernicious anemia, were present. Lichtheim pointed out both the similarity and the difference between this spinal lesion and tabes dorsalis. Minnich studied two cases in great detail and described, in one case, symmetric degeneration of the posterior columns, and in the other case, symmetric degeneration of both posterior and lateral columns of the spinal cord. He emphasized this condition as being distinctly different from tabes.

The authors who have summarized postmortem changes in cases of Diphyllobothrium anemia as being identical with those met with in cryptogenetic pernicious anemia, are, among others, Lichtheim, Minnich, Vierioujski, Eckert (109), Rosenqvist (354), Cramer, Rosenow (351), Warthin (464) and Faber (131).

PART III. PATHOGENIC MECHANISM AND TYPE OF ANEMIA

Theories regarding the pathogenic mechanism

Probably the first man to link together pernicious anemia and helminthiasis was Albrecht, pathologist at the Obuchow Hospital in St. Petersburg, who even before 1883 used to mark down in the proctocol of the necropsies under diagnosis of such cases, "Helminthiasis. Anaemia perniciosa." He apparenty had a conception of a close relationship between the two conditions, for in cases of cryptogenetic pernicious anemia he would make the notation: "Anaemia perniciosa. Tapeworms not found." Of the thirty cases of pernicious anemia which came under his observation in a decade, five were accompanied by infestation with *Diphyllobothrium latum.* However, this gave a frequency of occurrence of the two conditions together of 16.6 per cent. This was no higher than the frequency of the parasitism in the population of St. Petersburg at that time, which Klimenko gave as 15 to 20 per cent, and Sander as 25 per cent. Why a relationship was seen between the two conditions was not stated.

Almost simultaneously with Albrecht the famous Russian clinician Botkin (55) made the remark that he had observed several cases of pernicious anemia coinciding with the presence of *Taenia solium* or *Diphyllobothrium latum* in the intestines; he had also noticed that cure from the anemia was effected if a vermifuge had been successfully administered.

Hoffmann, in his lectures on general therapeutics, published in 1885, made the statement that he repeatedly had observed cases which he considered to be examples of pernicious anemia but in which he had seen recovery following anthelmintic treatment. He wrote:

> Here in Dorpat, where we are almost regularly dealing with *Diphyllobothrium latum,* we have gained the impression that this parasite is far from being harmless. I have collected a series of cases of people in middle age who have come to me with symptoms of severe anemia, and in such a

general condition as to make the prognosis very dubious. They improved in almost a miraculous manner after the tapeworms, which were found present, had been expelled. Such severe anemia is not a rare occurrence here. Previously I have been surprised at the great number of cases of pernicious anemia here. I am now convinced that a certain group of these cases cannot be regarded as pernicious anemia, but must be classified as tapeworm patients.

Although the two types of anemia, as far as Hoffmann could see, differed only in the absence or presence of the particular intestinal parasite under consideration, still he emphasized the distinction between them.

Botkin (56), in 1886, lightly referred to a case of grave chlorosis associated with *Diphyllobothrium latum* in a young girl who promptly recovered following expulsion of the parasite. No description was given; therefore, it is not known whether this case was one of chlorosis with low color index, or one of hyperchromatic anemia. Schauman's statement about coexistence of chlorosis and *Diphyllobothrium latum* without causal relationship has been referred to.

The first attempt at more detailed reports of cases was made the same year by Reyher at Dorpat in his publication of eleven successive cases of pernicious anemia and fish tapeworm in the same patients. All of these patients fully regained their health after expulsion of the worm. His descriptions are rather sketchy and therefore of questionable scientific value when it comes to determine the type of anemia involved. Prior to 1877 he had observed cases in which exactly the same syndrome had occurred and without exception the outcome was fatal. He was convinced that the patients whose cases were presented in this report would have died also if the parasites had been permitted to remain; some of the patients were moribund at the time of the first consultation. From his own observations and experience he found it not only possible, but rather plausible, that *Diphyllobothrium latum*, through its presence in the intestinal canal, might give rise to pernicious anemia. He also concluded that in this type of pernicious anemia the prognosis, with proper treatment, was good.

About simultaneously with Reyher, Mitzkuner, in St. Petersburg, reported a fatal case of pernicious anemia, in which necropsy revealed one *Diphyllobothrium latum* and two ascarides. He supported Botkin's (55, 56) conception that there is a causal relationship between the parasite and the anemia.

Runeberg (364), in 1887, reported his observations on a series of cases of pernicious anemia in Finland, between 1878 and 1887. From 1878 to 1883 nine patients died in his clinic at Helsingfors from pernicious anemia, most of whom, at necropsy, were found to have *Diphyllobothrium latum* in their intestines. Between 1883 and 1887 nineteen cases of pernicious anemia were treated at his clinic with only one death in hospital. The patient who died was moribund on admission. Four of the patients had cryptogenetic pernicious anemia. One of these four patients had died, in an exacerbation at home; two were having exacerbations at the time of the report, and the fate of the fourth was unknown. In fourteen cases, some etiologic factor seemed plausible. Twelve patients harbored *Diphyllobothrium latum*, one, *Ascaris lumbricoides*, and one was pregnant. In the remaining case the etiology was undetermined. Runeberg's study revealed the presence of fish tapeworm in 63 per cent of his cases of pernicious anemia. As controls he examined the stools of fifty-two nonanemic patients in his clinic for ova of Diphyllobothrium and found infestation in 13.5 per cent. He therefore concluded that the parasite somehow played a part as an etiologic factor, and could not be regarded merely as a coincident resident.

Lichtheim, in 1887, reported two cases of pernicious anemia associated with *Diphyllobothrium latum*, both from Bern. One was typical, the anemia was profound, and the patient died a few days after admission. In this case Lichtheim observed a lesion of the spinal cord that in some respects was similar to tabes dorsalis. The other was complicated by arteriosclerosis; the anemia was less advanced and responded slowly yet favorably to anthelmintic treatment. The prevailing ignorance concerning the etiology of pernicious anemia, he felt, placed the definition of the disease on a symptomatic and morphologic basis. Pernicious anemia he considered to be a clinical and hematologic entity. Litten (245), in the discussion that followed Lichtheim's reading of this paper, professed the same viewpoint.

From St. Petersburg several cases of Diphyllobothrium anemia were reported in 1888. Schapiro described a typical case in a boy aged thirteen years. Immediate and extremely rapid recovery resulted from expulsion of the parasite by a vermifuge, and seven to eight weeks later he had erythrocytes in normal number. The color index was high. Podwissotzky also observed a typical case in a boy, aged fourteen years, but was hesitant in expressing any opinion as to the significance of the parasite as a specific producer of pernicious anemia. Kissel, reported the case of a boy aged six years (the youngest on record), who died with all essential symptoms of pernicious anemia four days after his admission to the hospital. Necropsy revealed the presence of the fish tapeworm. Vieroujski and Shimanskiy each reported one case.

Mueller, in 1889, briefly referred to a fatal case which he had observed in Berlin. He did not doubt the significance of *Diphyllobothrium latum* as an etiologic factor in the development of grave, even fatal anemia, and found no serious objection to this viewpoint in the fact that a certain percentage of the patients will die even when the worm is expelled, for "when the anemia once has reached a very high degree, it may progress on its own momentum as an independent disease."

Between 1889 and 1891 Runeberg (365, 366) reported seven additional cases, with two deaths and five recoveries. Westphalen (470, 471) reported two cases which responded slowly yet favorably on anthelmintic treatment. Dehio (99) emphasized the high color index in his five cases.

Additional articles were published by Ravitsch-Shtcherbo and by Dehio (101), each of whom reported one case, and by Winiarski, who reported three cases. Minnich recorded two minutely studied cases in which the spinal cord was involved, one of which may be the case to which Lichtheim referred in 1887. Both of Minnich's cases terminated fatally and the diagnosis was verified at necropsy.

Pariser and Eckert (109) each added another case to the series, and Wiltschur, in a paper dealing with the pathogenesis of pernicious anemia, mentioned that he had seen twelve patients with Diphyllobothrium anemia in his practice, all of whom had been cured by proper anthelmintic treatment.

Until 1894 the publications concerning Diphyllobothrium anemia had been rather casual, and were based on too scant material definitely to establish the type of the anemia in question and to allow study of the tapeworm in its relationship to the development of this anemia. The majority of the writers up to that year were inclined to assume that the broad tapeworm, under certain circumstances, might lead to grave, even fatal, anemia. Differences of opinion were voiced, and in the main concerned the identity of this form of anemia with that of Addison-Biermer. Only Podwissotzky questioned the causal relationship between the parasite and the changes in the blood. Other writers, who doubted the alleged etiology in Diphyllobothrium anemia, but who had not personally observed such a case, were Heller, Roux (358), who found ninety fish tapeworms in a perfectly healthy girl; Reymond, who suggested that the occurrence of pernicious anemia and *Diphyllobothrium latum* was coincidental, and Kroenig, who had noted the tremendous frequency of infestation with fish tapeworm in eastern Prussia, with apparently no higher mortality there than in places where the parasite is rare.

A great stride in knowledge of this disease was instigated by Schauman's monograph (381) in 1894. He contributed seventy-two reports of cases; thirty-six of the cases had been personally observed by the author. By his thorough and scientific investigation a solid foundation was laid for further researches. He demonstrated beyond doubt that *Diplyllobothrium latum* played an active part in the pathogenesis of the particular anemia under investigation. He pointed out that although most of the patients with Diphyllobothrium anemia would die if the parasite was permitted to remain, after administration of a suitable vermifuge as a routine the mortality rate had dropped significantly and the prognosis was doubtful only in very advanced cases, as a rule.

The usually rapid and prompt recovery following expulsion of the parasite, and the usually permanent cure, seem sufficient proof of the causal connection. Only a few have voiced opinions contrary to this viewpoint. Heller's suggestion has been alluded to earlier. The probability of occurrence of cryptogenetic pernicious anemia and coincidental infestation with tapeworm has been offered by Warfvinge, Shiperovich, Roux, Reymond, and Kroenig, but the latter three had not personally seen a case of Diphyllobothrium anemia. The possibility of such coincidence was mentioned by Isaacs, Sturgis and Smith.

But why is it that anemia develops in only a very small proportion of carriers of *Diphyllobothrium latum*, and the great majority enjoy good health? The cause of this peculiar fact may be seen in relation to either the parasite or the host. Some questions might be formulated and answered, which would serve as a review of the theories and discussions regarding the part played by the parasite.

The parasite. In the first place, are there any indications that the quantity of worm contained in the intestines is significant? Many times, huge numbers of worms have been observed in healthy persons (175, 358). On the other hand, the majority of patients with anemia harbor very few of them, or only a single worm, and in a number of cases a worm could not be demonstrated at all, despite the presence of a number of ova in the stools before death and in the intestinal content after death. Neubecker called this condition "Bothriocephalusanämie ohne Bothriocephalen" or Diphyllobothrium anemia without Diphyllobothrium.

Is it, then, the duration of the infestation which decides whether or not a carrier is going to develop anemia? This question is not easy to answer, owing to the difficulty in establishing the duration of infestation. Passage of segments in the stools may take place so infrequently and inconspicuously that the host is unaware of the presence of the parasite. The problem of spontaneous expulsion, a long free interval, and reinfestation, is also to be considered. Vlayeff, Askanazy, and Bard stressed the duration of infestation as a significant etiologic factor, but there is much evidence in disagreement with their view. Most writers do not attach much significance to the factor of time. Decades of infestation, without any sign of ill effect on the host, have been reported time and again (267, 374), and less than one year has been found sufficient time to cause anemia to develop (341).

The question has also arisen whether it may be the condition of the parasite which has something to do with the anemia. The morbid effect has been attributed to disintegration, death, and disease of the whole or part of the parasites (17, 87, 96, 100, 109, 114, 155, 170, 300, 310, 351, 380, 477, 478). Instances of Diphyllobothrium anemia without Diphyllobothrium have been recorded by Dehio (100), Westphalen (471), Podwissotzky, Sander, Neubecker, Ragosa, Schauman (381–383), Winiarski, Rosenqvist (354), Vierioujski and Eckert (109). It has been seen that an anemic patient who has had numerous ova of Diphyllobothrium in the stool, before a vermifuge has been administered, has improved after taking a vermifuge and after the ova have disappeared from the feces, without any visible evidence of expulsion of the worm. Or the condition of the patient may have been too poor and the anemia too far advanced for a cure to be effected by a vermifuge. In such a case, ova may have been plentiful in the stools before death and in the intestinal content at the time of the necropsy, but a worm may not have been found.

This fact is thought to be due to excessive disintegration of the whole worm. The disintegration is considered to be the cause of the anemia by many writers, but when a similar condition of the worm may be seen in nonanemic carriers as well (25, 261, 382), then a basis for such a contention is obviously lacking.

Disintegration of retained proglottids, which in many instances are expelled only at infrequent intervals, even though the worm itself remains healthy, has been considered the cause by other authors. And still other writers have explained the findings of a sound looking parasite in an anemic patient by assuming, without proof, that one or more additional worms must have been present and undergone disintegration. Tallqvist (430) found that worms from anemic patients, in the majority of cases, gave evidence of loss of substance, either by shrinkage or dissolution of proglottids. Saltzman (372) stated that in the majority of the cases of Diphyllobothrium anemia, the worm appeared to be pathologically changed. He seemed to think that "the increased decomposition of the worm which so often is observed in Diphyllobothrium anemia patients, in some way or other may be caused by dominating factors existing together with the anemic condition of the body, and that the decomposition may decrease and end when the condition of the patient improves." Becker (25) also has touched on this conception and has stated that if the parasite shows a tendency to disintegrate in a case of far advanced anemia, this occurrence might just as well be a result of the anemia as a cause of it.

Individual variation in condition or toxicity of the tapeworms was suggested by Leichtenstern, Lenhartz, Schapiro, Lazarus, and Grönberg (162). Murata and Küchenmeister offered the suggestion that fish tapeworms in hosts in whom anemia developed might be of a different species than worms which did not cause anemia. And Schmaltz attributed the variable pathogenicity to geographic peculiarities.

Warfvinge and Shiperovich expressed the belief that the worm, through its presence in the intestine, predisposes the organism of the host for attack by an unknown factor. Neubecker and Galli-Valerio considered the balance of the virulence of the parasite as against the "resistance" of the host.

The worm, living in and being nourished by the intestinal content, may deprive the host of an important nutrient material which is necessary for the maintenance of the equilibrium of the blood. Reyher, Vlayeff, and Willson (475) have theorized on this possibility. Reyher and Litten (246) also suggested the possibility of the worm favoring invasion by microörganisms. Here we may again ask ourselves how the quantity of worm contained in the intestine and the duration of infestation can be unimportant.

It is well known that there is a certain amount of gastro-intestinal disturbance associated with helminthiasis. Winiarski has expounded the idea that tapeworms may cause pernicious anemia only indirectly, by way of gastro-intestinal upsets, and that these may be considered the real cause. However, there is great variability of these disturbances, and although they must be attributed to the presence of the parasite, no one has attempted to prove that the anemia depended on the degree, extent or duration of these gastro-intestinal disturbances. Many carriers may suffer from periodic diarrhea, constipation, or alternating diarrhea and constipation without showing signs of anemia, and, on the other hand, such disturbances are often absent in cases in which Diphyllobothrium anemia develops.

Botkin (55, 56), Mitzkuner, and Runeberg (364, 365) adhered to the theory that the worm exerts incessant irritation on the intestinal mucosa and that the impulses are then carried to the hypothetic blood centers in the medulla oblongata, which influences the composition of the blood by controlling the production of erythrocytes. That some influence exerted by way of the nervous system may be at least partly responsible for the disturbance of the blood is a possibility which cannot be excluded entirely (228, 475). Of course, an explanation is not given for the fact that only a small number of the carriers react to the supposed stimulation with alterations of the blood.

Most writers, however, adhere to the theory that toxins cause the disturbance, in conformity with the generally accepted hypothesis of the pathogenesis of cryptogenetic pernicious anemia. Whether disintegrated, dead, sick or healthy, the parasite is supposed to yield a toxic material, exotoxin or endotoxin, which, when absorbed into the blood stream, has a profound effect on the composition of the blood (14, 18, 69, 87, 96, 97, 100, 101, 109, 114, 170, 196, 203, 284, 300, 310, 351, 354, 367, 369, 380, 381, 437, 477, 478).

Of what nature is this toxin? Many have tried to solve this problem, and a great deal of research work has been carried out to prove theoretic speculations. In most of this work, various tapeworm substances have been injected intravenously into experimental animals and resulting changes in the blood have been studied.

Silbermann first showed that chronic anemia could be produced experimentally by employing different chemical substances such as pyrogallic acid, pyrodine and others, without any evidence of acute intoxication. He also pointed out the great similarity between the pathologic condition produced, and pernicious anemia. Silbermann's work was repeated by von Voss in an attempt to find out whether changes occurred in the spinal cord in the course of experimental anemia. The anemia was not typical of pernicious anemia. Pathologic changes in the cord were found in but one of the animals, and in that one case it was not characteristic of subacute combined sclerosis. Tallqvist (427) gave pyrogallic acid and pyrodine to dogs by mouth, in increasing doses, and succeeded in producing progressive, destructive anemia, with microcytes, macrocytes, polychromatophilia, color index greater than one, erythroblasts, hemosiderosis of internal organs, and hyperplastic bone marrow. Comparing these observations with those in pernicious anemia and Diphyllobothrium anemia he found strong support for the conception that both Diphyllobothrium anemia and pernicious anemia have toxic etiology.

Vlayeff was the first to experiment on the toxicity of the fish tapeworm. He made extracts, and administered them to rabbits and a pigeon; the results were entirely negative.

Schauman and Tallqvist gave to small adult dogs increasing doses of preparations of *Diphyllobothrium latum*. By mouth he gave worm digested by trypsin, saline emulsion of worm, or pieces of substance of worm in amounts of 30 to 50 grams daily. Percutaneously he gave saline solution of the worm, run either through cotton or through a

Chamberlain filter. All of these preparations gave similar results, regardless of whether they were made from worms that had been harbored by healthy or by anemic persons. In all of the dogs, more or less marked hyperchromatic anemia developed. Similar results were obtained by Schmauch with a saline solution of worms injected intraperitoneally into cats. Galli-Valerio briefly stated that extract of macerated worms found in anemic patients had a stronger hemolytic power than extract of live worms.

These preliminary experiments indicated the presence of some hemotoxin within the body of the tapeworm and further investigations became necessary to attempt to isolate the active fraction.

A thoroughly conducted study to this end was undertaken by Tallqvist (429, 430). He found the body of the worm to contain (*a*) a strong proteolytic ferment which rapidly caused autolysis of the worm; (*b*) an antifermentative substance, which protected the parasite from digestion by pepsin and trypsin; (*c*) a hemagglutininic protein, and (*d*) a hemolytic lipoid which comprised about 10 per cent of the total substance of the dried worm. This hemolysin was active both in vitro and in vivo, in dogs and in rabbits, and whether given intravenously, subcutaneously or by mouth. It produced in the animals, mild, but definite, hyperchromatic anemia, with normal or diminished leukocyte count, associated with a tendency to relative lymphocytosis. The course was characterized by remissions. The general condition of the animals suffered little. Postmortem examination revealed hemosiderosis of the liver and spleen, hyperplasia of the bone marrow, and fatty changes of the cardiac muscle.

The author also experimented on himself by taking the lipoid by mouth in increasing doses. His weight and general condition remained unaltered during the time of the experiment. The mean results of examination of the bood before the start of the experiment were as follows: erythrocytes 5,577,000; hemoglobin 78.2 per cent, color index (corrected) 1.00, and leukocytes 7,600. Taking of Diphyllobothrium lipoid for sixteen days in doses increasing from 0.06 to 0.40 grams daily, reduced the number of erythrocytes to 4,140,000 and the hemoglobin to 75.5 per cent, and raised the color index (corrected) to 1.30 and the leukocytes to 8,400. One week after the administration of lipoid was discontinued the blood was approximately in the state in which it had been before the experiment started.

It might be of interest to insert here that Tallqvist, who devoted much of his time to the study of anemia, about twenty years later himself became a victim of cryptogenetic pernicious anemia.

Other types of tapeworms that were analyzed did not appear to contain a hemolyzing substance (427). However, hemolytic substances could be extracted from several normal organs, especially from the mucosa of certain parts of the gastro-intestinal tract (40. 41, 427).

The next step was to determine the nature of the lipoid substance, and Faust and Tallqvist tested every fraction of this substance as to its hemolytic properties. They found, as an end result, that hemolysis depended on the quantity of oleic acid present. The unsaturated acid itself, its sodium salt, and its cholesterol ester, had a strong hemolytic effect, although not as strong as the original Diphyllobothrium lipoid. Normal serum was shown to have an inhibitory effect on hemolysis in vitro. A certain concentration of the cholesterol ester of oleic acid was necessary before any harmful influence on the erythrocytes could be demonstrated. Three hours after a dog had been fed Diphyllobothrium lipoid by tube, a fistula of the thoracic duct was established and the chyle was collected for the next five hours. This chyle did not contain cholesterol, whereas free fatty acids, with strong hemolytic properties, were found. To explain this, the investigators assumed that the cholesterol esters in the food are broken down in the intestinal tract, the free fatty acids combining with alkali, whereas cholesterol passes out quantitatively. The fatty acids are then resorbed as soaps. If oleic acid is involved, this is supposedly the agent responsible for eventual destruction of blood.

Faust and Schmincke carried on experiments in order to find out how a continuous intake of oleic acid would affect the animal organism, more specifically the blood. They met with disappointment, inasmuch as daily doses of 10 to 25 grams effected only moderate diminution of the content of hemoglobin, whereas cell counts and smears appeared normal. Schmincke and Flury found that depression of the hemoglobin was purely temporary and that feeding for sixteen months of 10 grams daily of oleic acid had no effect whatever on the condition of the blood of a small-sized adult dog. The organism seemed to develop a protective mechanism against the erythrotoxic effect of the oleic acid and this was expressed by increased resistance, of specific nature, against the hemolytic agent.

Faust and Schmincke produced hypochromatic anemia (secondary type) in rabbits by subcutaneous injections of oleic acid.

It is evident, therefore, that oleic acid is not the specific or the important factor in the production of pernicious anemia in Diphyllobothrium carriers.

Boycott (59), Bondouy, Csonka, Beumer and Beumer and Bürger attached little, if any, significance to oleic acid as a cause of anemia. Meyerstein produced hemolysis and hemoglobinuria (oxyhemoglobin in the first portion of the urine; methemoglobin later) in rabbits by intravenous injection of tremendous doses (2 to 20 cc. of 1 per cent of sodium oleate in physiologic solution of sodium chloride). Sodium stearate and palmitate had similar effect. Cholesterol inhibited hemolysis caused by soap; still, he concluded that soaps, when present in abnormally high quantities, due either to endogenous formation or to alteration of the resorption of fats, may take active part in the development of hemolytic anemia. K. Meyer (273) found that the serum protein also had an inhibiting effect on hemolysis of soap in vitro.

Weinberg (466), classifying the hemolysins isolated from the broad tapeworm with the group that had been isolated from normal tissues, the so-called tissue lysins, was disinclined to attach any etiologic significance to these fractions. However, he vigorously defended the theory that a toxin which causes hemolysis is active in hookworm anemia.

At this point the investigations into the toxicity of *Diphyllobothrium latum* had seemingly come to a standstill; the originally suspected lipoid (oleic acid) was declared rather harmless by the majority of the research workers.

Seyderhelm (403, 404), in 1918, published some interesting observations. In collaboration with his father he began a study of a condition variously called pernicious anemia in horses, swamp fever in horses and anémie infectieuse des chevaux. This condition differed considerably from pernicious anemia in man, particularly in that it was transmissible by injecting a normal horse with blood from a sick animal. Megaloblasts were never seen in the blood, and myeloid metaplasia in liver and spleen were found to a degree comparable only to that in myelogenous leukemia in man. These investigators succeeded in demonstrating a causal relationship between this swamp fever and the presence of gastrophilus larvae (*Oestrus equi*) embedded in the wall of the stomach of the sick horses. An aqueous extract of these larvae proved to be highly toxic, the extract of two or three of them being sufficient to kill a horse when injected intravenously. Given in small, repeated doses, swamp fever developed, identical with the genuine type, and furthermore, this experimentally produced anemia could be transmitted to sound horses in the same manner and in them lead to a fatal outcome. The toxic effect was laid to the so-called oestrin fraction, which is present not only in larvae taken from horses suffering from swamp fever but also in larvae from healthy horses. Purified lipoid from the larvae produced only moderate secondary anemia in rabbits when injected intravenously in successive doses, and there was a strong tendency to restitution; to obtain anemia of this grade an amount of lipoid corresponding to 100 and more larvae was required. The oestrin, however, had strong erythrotoxic, neurotoxic, and pyrogenic potency, although in vitro it did not produce hemolysis. A dose of 0.00008 gram was fatal to a horse weighing 400 kgm. The anemia produced by successive injections of sublethal doses over a period of time was characterized by hyperchromasia and polychromasia, anisocytosis, poikilocytosis, increased resistance of erythrocytes, a decreased platelet count and leukopenia, and myeloid metaplasia in liver and spleen. Oestrin-produced experimental anemia, furthermore, could be transmitted to a healthy rabbit by injection of blood of the horse used in the experiment. Ess and Schalk, and Marxer confirmed Seyderhelm's results with oestrin but were inclined to believe that they were dealing with anaphylactic phenomena and not with the genuine infectious anemia.

Seyderhelm became interested in the problem of whether or not the same principle was active in the production of pernicious anemia in human beings. Following the same technical procedure with which he isolated oestrin from *gastrophilus larvae*, he succeeded in preparing a similar substance from *Diphyllobothrium latum*, bothriocephalin. His results with this fraction justify a description of its isolation: (*a*) the worms, ground to a homogenous mass and mixed with quartz sand, were macerated in 50 per cent alcohol; the mixture was shaken for hours in the course of which the alcohol was changed several times; (*b*) the portions of alcoholic extract were added together and filtered; to the filtrate was then added, drop by drop, absolute alcohol in equal amount or twice that much; (*c*) in the next twenty-four hours, a white precipitate formed which was filtered through a hard filter, and the residue was washed on the filter with 90 per cent alcohol; (*d*) the filter, with the residue, was shaken in distilled water, whereby the residue would go into solution; (*e*) the precipitation with strong alcohol was repeated twice, and the final residue was dissolved in distilled water; (*f*) the watery solution was then added, drop by drop, to absolute alcohol until a concentration of 80 per cent alcohol had been obtained. The precipitate which formed in twenty-four hours was filtered off on a hard filter, dissolved in distilled water, evaporated to dryness, and finally redissolved in physiologic solution of sodium chloride.

Employed in experiments on rabbits, bothriocephalin was found to be highly toxic, and to have an effect similar to that obtained with oestrin, except that anemia experimentally produced by bothriocephalin was not transmissible. Repeated intravenous injections of small doses of bothriocephalin produced progressive, in the end fatal, hyperchromatic anemia, with anisocytosis (macrocytes), poikilocytosis, polychromasia, normoblasts in large numbers, and occasionally, megaloblasts; initial leukocytosis changed into leukopenia. Necropsy revealed extramedullary formation of blood in the liver and the spleen, and hemosiderosis, but yellow bone marrow.[3] Bothriocephalin had no hemolytic effect in vitro. It was present in worms taken from anemic as well as from healthy carriers.

Becker, Helander and Simola repeated and extended Seyderhelm's work on bothriocephalin. They confirmed that it was nonhemolytic in vitro, but they also found that it became hemolytic in vitro if normal human blood serum was added to it. Bothriocephalin prepared from different worms seemed to vary in toxicity, and different worms yielded different amounts of the substance, which on standing appeared to lose toxicity. They produced in rabbits, by intravenous injections of increasing doses, moderate hyperchromatic anemia, polychromasia, normoblasts, a tendency to leukocytosis, slight hemosiderosis of the liver and spleen, and hyperplastic bone marrow, and they saw great similarity in effect between bothriocephalin and the so-called chemical hemotoxins.

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[3] The statement that the bone marrow was yellow is unexpected and does not agree with Becker, Helander and Simola who, in repeating Seyderhelm's work, found the bone marrow to be hyperplastic.

Nyfeldt isolated three fractions of seven worms taken from nonanemic carriers in the following way: One worm had been left alone for three days and had changed into a milky liquid through autolysis. After dilution with physiologic solution of sodium chloride it was run through several layers of filter paper. To the opaque filtrate under vigorous shaking was added an equal quantity of absolute alcohol. A grayish precipitate formed which was filtered off on a hard filter, washed several times with absolute alcohol, then several times with ether, and finally dried by suction of air through the filter at room temperature. The dry residue was dissoved in physiologic solution of sodium chloride, standardized (as explained later) and labeled Bothriotoxin I. The clear alcoholic fluid which had been filtered off from the precipitate mentioned was then measured, and the double volume of absolute alcohol was added. In addition, sufficient ether to make about 10 per cent of the total volume and acetic acid of 50 per cent concentration to make about 1 per cent of the total volume were poured into the alcoholic solution. In the course of twenty-four hours a white precipitate formed; this was filtered off on a hard filter, was washed with several portions of absolute alcohol, and then ether, and finally was dried by suction with air. The dry residue was dissolved in physiologic solution of sodium chloride and was standardized, and labeled Bothriotoxin II. Six worms, preserved for three days under toluol, served as material for the preparation of bothriocephalin after the manner of Seyderhelm, which also was standardized.

The bothriocephalin solution gave the chemical reactions of albumoses.

Standardization of the solutions was done in the following way: Stock solutions were made so dense that they had to be diluted in order to match standard 10, which in density corresponded to a bacterial emulsion of 1,000,000,000 bacteria to 1 cc. The scale of standards ran from 5 to 15, corresponding in density to a bacterial emulsion of 500,000,000 to 6,000,000,000 bacteria in 1 cc.

None of the three fractions had hemolytic effect in vitro, whereas they all produced hemolysis in vivo. With bothriotoxin I and II in rapidly increasing daily doses Nyfeldt succeeded in producing hemolytic, hyperchromatic, megalocytic anemia with leukopenia, whereas in the experiments with bothriocephalin the regeneration kept pace with the destruction, just as was seen when bothriotoxins I and II were given in constant dosage. Table 20 exemplifies the progress of the anemia.

TABLE 20

Progression of anemia in a rabbit given daily injection of increasing doses of Bothriotoxin I. (Nyfeldt)

SEPTEMBER	DOSE	ERYTHROCYTES	DIAMETER OF ERYTHROCYTES			HEMOGLOBIN	COLOR INDEX		RETICULOCYTES	LEUKOCYTES	PER CENT				
			Smallest	Largest	Mean		Direct	Corrected			Lymphocytes	Monocytes	Neutrophils	Eosinophils	Basophils
		millions	*microns*	*microns*	*microns*	*per cent*			*per cent*						
15	0.5 cc. Standard 5	5.48	5.25	8.25	7.33	80	0.73	1.00	1.5	11,760	71.25	4.75	20.25	3.75	0.00
20									14.5						
23	1.0 cc. Standard 10	2.73	3.75	9.75	7.56	49	0.89	1.20		4,710	83.25	2.75	7.00	6.25	0.75
27	3.0 cc. Standard 12	1.50	3.00	13.50	8.87	41	1.37	1.88	31.0	3,670	96.50	0.25	2.50	0.75	0.00
29	Animal died														

Busson and Kosian expressed doubt as to whether or not the artificially obtained hemotoxins play a part in the pathogenesis of diseases of human blood.

Owing to the fact that infestation with *Diphyllobothrium latum* has been found greatly concentrated in a relatively small area around the Baltic Sea, this parasite has become very conspicuous as an etiologic factor in the development of the syndrome of pernicious anemia, whereas the significance of other intestinal parasites in this respect has faded into the background. The syndrome of pernicious anemia, however, may be precipitated by other parasitic influences as well.

Poggenpohl and von Jaksch referred to Taeniae as being capable of producing pernicious anemia. Vieroujski used the general term "tapeworm" in the report of his case, without specifying the type.

Table 21 shows instances of simultaneous occurrence of pernicious anemia with intestinal parasites other than *Diphyllobothrium latum*. The list is as complete as the literature has been available; the anemia is not in every case reported in such detail that the diagnosis pernicious anemia was completely convincing, but sufficient evidence was offered to make the diagnosis probable. In a series of reports (13, 20, 69, 104, 311, 315, 322, 368, 466), the data furnished were either insufficient to suggest this form of anemia or strongly in favor of a hypochromatic type of anemia. Although Naegeli (296, 297), Boycott (58), Guidi, Liermberger, Christoffersen and others, and also most textbooks categorically have stated that the anemia associated with blood suckers is always secondary in type, Ferguson observed that in Egypt, in a minority of cases of ankylostomiasis a blood picture which closely resembled pernicious anemia was presented. Ferguson explained this on the ground that the worms caused multiple small lesions of the intestinal mucosa, and paved the way for invasion by septic material. Inouye observed that a certain percentage of patients with anemia due to *Fasciola hepatica* had a hyperchromatic type of anemia; the color index sometimes was as high as 2.0. Gluzinski reported such a case in detail; the clinical picture was rather mixed, however, due to obstructive jaundice. Lazarus contended that anemia in ankylostomiasis has a double pathogenesis: (a) withdrawal of blood, which causes posthemorrhagic, secondary anemia, and (b) resorption of toxic-hemolytic substances from the intestinal content, which causes toxic-hemolytic, primary anemia. If one of these factors was predominating, the type of the anemia in the individual case would be determined.

Multiple infestation has been recorded. Babes reported *Diphyllobothrium latum* and *Taenia solium*; Mitzkuner and Shiperovich, *Diphyllobothrium latum* and *Ascaris lumbricoides*; Sievers and von Bonsdorff, *Diphyllobothrium latum*, *Lamblia intestinalis*, and *Balantidium coli*; Sahli (368), *Anchylostoma duodenale*, *Anguillula intestinalis* and *Trichocephalus dispar*; Pariser, *Diphyllobothrium latum* and *Taenia saginata* (type of anemia not given); Aronade, *Ascaris lumbricoides*, *Oxyuris vermicularis* and *Trichocephalus dispar* (type of anemia not given);

TABLE 21

Pernicious anemia associated with intestinal parasites other than Diphyllobothrium latum

AUTHOR	PARASITE	CASES	RESULT	REMARKS
Riess	*Taenia solium*	?	?	
Babes	*Taenia solium*	1	Cured	*Diphyllobothrium latum* also present
Bloch (50)	*Taenia solium*	2	?	
Botkin (55, 56)	*Taenia solium*	Several	Cured	
Dervis	*Taenia solium*	1	Cured	Reëxamined four years later
Becker, E. (23)	*Taenia saginata*	1	Dead	
Schreiber	*Taenia saginata*	1	Cured	Color index varied between 0.58 and 1.67; reëxamined 6 and 16 (Naegeli) years later
Reckzeh	*Taenia saginata*	1	Dead	Three months after expulsion of worm, following temporary improvement
Andree	*Taenia saginata*	1	?	
Fiedeldij	*Taenia mediocanellata*	1	Cured	
Nonne	*Taenia mediocanellata*	1	Dead	Complicated by subacute combined sclerosis
Eisenlohr	*Taenia mediocanellata*	1	Cured of anemia	Spinal cord lesion not affected by expulsion of worm
Saltzman (374)	*Taenia mediocanellata*	1		*Diphyllobothrium latum* also present
Rossi	*Hymenolepis nana*	2	Cured	
Demme	*Ascaris lumbricoides*	?	Cured	
Gomess	*Ascaris lumbricoides*	5	?	The parasites were infested with gregarines
Sakheim	*Ascaris lumbricoides*	1	Dead	
Vierioujsky	*Ascaris lumbricoides*	1	?	
Runeberg (364)	*Ascaris lumbricoides*	1	?	
Mitzkuner	*Ascaris lumbricoides*	1	Dead	*Diphyllobothrium latum* also present

Karvonen	*Ascaris lumbricoides*	1	Cured	
Solley	*Ascaris lumbricoides*	1	?	
Savolin (377)	*Ascaris lumbricoides*	2	Cured	Reëxamined twelve and seven years later, respectively
Shiperovich	*Ascaris lumbricoides*	1	Cured	*Diphyllobothrium latum* also present
Bardenwerper	*Ascaris lumbricoides*	1	Dead	
Barker (18)	*Ankylostoma duodenale* (necator or uncinarius Americanus)	1	Cured, or at least improved	Considered by author a coincidence
Ferguson	*Ankylostoma duodenale*	Several	?	A certain proportion of patients with hookworm in Egypt presented a pernicious type of anemia
Allan	*Ankylostoma duodenale* (necator or uncinarius Americanus)	1	Cured	Reëxamined four years later
Hemsen	*Anguillula intestinalis*	1	Dead	
Runeberg (364)	*Oxyuris vermicularis*	1	?	*
Swan	*Trichomonas intestinalis*	1	Dead	
Savolin (378)	*Lamblia intestinalis*	2	Improved	Under observation
Sievers and von Bonsdorff	*Balantidium coli*	1	Improved	*Diphyllobothrium latum* and *Megastomas entericum* also present
Jennings	*Balantidium coli*	1	Improved	Parallelism between degree of anemia and degree of infestation, exacerbations and remissions in later course
Logan (248)	*Balantidium coli*	4	2 dead†	In three of the cases a familial tendency towards pernicious anemia seemed to exist

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* Runeberg (364) stated in Finska Läk-sallsk. Handb. that his patient was infested with *Ascaris lumbricoides*, whereas in Deutsch. Arch. f. klin. Med. he called the parasite *Oxyuris vermicularis*. In both papers he seemed to be referring to the same patient.

† The other two died later; three additional patients have since been observed in The Mayo Clinic (249).

Saltzman (374), *Diphyllobothrium latum* and *Taenia mediocanellata*. In Stukowski's case the association of chronic myelogenous leukemia, *Diphyllobothrium latum* and *Taenia saginata* was in all likelihood pure coincidence.

The problem of the toxicity of intestinal parasites other than *Diphyllobothrium latum* has intrigued many investigators. Tallqvist (430), Boycott (57), Guerrini, Cao, Jammes and Mandoul, LeDantec, Bernabo, Bussano, Lynch, Blanchard, Whipple and others claimed that toxicity was absent, whereas Calamida, Messineo and Calamida, Mingazzini, Pomella, Phillips, Bedson, Brian, Paulian, Rachmanow, Petri, Weinberg (465), Schimmelpfennig, Flury, Schwartz (400), Seyderhelm (402), and others found strong evidence for such toxicity. Ragosa felt that whatever pathogenic property the Diphyllobothrium possessed, it shared with the Taeniae. Fetterolf found an antienzyme, in *Taenia saginata*, which had an inhibiting influence on enzymatic activity on starch and fibrin.

The divergence of experimental results and opinions may be due, in part, to differences in technical procedures, in ways of approaching the problem and in modes of reaction in the animals (57, 308).

The question naturally arises whether tapeworms, other than *Diphyllobothrium latum*, possess a specific property for precipitating anemia, or whether, in these cases, the coincidence of cryptogenetic pernicious anemia and of the particular parasitism is only a matter of chance. Although a great many of the patients infested with parasites other than *Diphyllobothrium latum* died from the anemia, a few reacted to successful anthelminthic treatment in a way seen as typical in Diphyllobothrium anemia. Dervis, Schreiber, Naegeli (296), Savolin (377) and Allan reviewed their cases four to sixteen years afterward and noted no relapse of the anemia. Ehrström (118–120) has shown that pernicious anemia does not occur with much greater frequency among Diphyllobothrium carriers than among carriers of other intestinal parasites. The great number of cases of Diphyllobothrium anemia in Finland is a direct result of the prevailing extensive infestation. The low incidence of infestation by other intestinal worms in northern Europe explains the rarity of anemic complications in those cases. Ehrström did not see an essential or principal difference in the effects of the various kinds of intestinal parasites; he saw only the difference that one kind might cause pernicious anemia more frequently than others. Ehrström could not find convincing proof of toxic resorption taking place in instances of anemia. To him, it seemed rather strained to assume that the different types of parasites produce the same toxin, or that they produce different kinds of toxin with identical effects on the host. The parasites have one thing in common, their localization in the intestinal tract. This, after all, may be a feature of significance. The discussion of the pathogenesis of cryptogenetic pernicious anemia has also centered in the gastro-intestinal tract (120, 131, 189, 367). Glossitis, achlorhydria, and diarrhea are well known associates. In sprue the condition of the blood often resembles that in pernicious anemia. In patients and experimental animals with intestinal strictures pernicious anemia has been known to develop. Also, in a few instances pernicious anemia has followed gastrectomy and resection of part of the small intestine and sometimes it has been seen in association with benign and malignant tumors of the stomach or colon.

A variety of gastro-intestinal disturbances has thus been claimed to be capable of producing pernicious anemia. The influence of intestinal parasites, therefore, appears more likely to be nonspecific. Although their significance is more or less generally admitted, it is also realized that only secondary importance can be ascribed to them (331). At most, they are inciting, releasing and precipitating in nature.

The carrier. The frequency of anemia in Diphyllobothrium carriers in Finland is variously estimated at from 1 in 659 (402), to 1 in 5000 or 1 in 10,000 (118). The worm itself possesses insufficient pathogenic potency to explain the occurrence of the anemia.

It seems reasonable, therefore, to assume the existence of individual susceptibility toward exogenous influences, such as that created by the presence of the fish tapeworm. This suceptibility may not involve the whole body; it may affect only certain organs or systems of organs.

The part played by the gastro-intestinal tract in the pathogenesis of the anemia is as yet undetermined. Seyderhelm (403) considered the intestinal wall to be a protective barrier against possible toxins contained in the intestine, and that the breaking down of this barrier was a necessary condition for the absorption of the toxins; the presence of the tapeworm may suffice to bring about this breakdown. Becker (25) concluded, from his own and Ragosa's work on the blood picture in nonanemic carriers, that certain toxins are continuously entering the blood of all carriers, even in cases in which anemia has not developed, a thought which Schauman (382) had mentioned fifteen years earlier. Willson (475) theorized on the worm interfering with the function of the intestine, chiefly with its supposed production of secretions necessary for the well-being of the blood-forming organs. He conjectured, also, concerning the nature of this interference; whether it is mechanical, reflex, toxic or combined. Another factor that may enter into the cause of the anemia is the hemoglobin metabolism. Abderhalden determined that destruction and replacement of hemoglobin is in the amount of about 12.5 grams in twenty-four hours in a normal adult, corresponding to about 3.5 per cent of the total number of erythrocytes. As to the mechanism by which this hemoglobin metabolism is regulated and stabilized, Ehrström (117) tentatively adopted the current hypothesis that a hormone may be in control. He intimated that the seat of production of this hypothetical hormone may be somewhere in the gastro-intestinal tract. He mentioned the possibility of three main conditions which might depress or extinguish the supposed action of the hormone: (*a*) the formation of the hormone may be decreased or absent; (*b*) its action may be rendered difficult or impossible through unfavorable alterations in the chemical milieu, and (*c*) the hormone may undergo chemical changes or may be destroyed by the supposedly altered chemical milieu.

The possibility of endocrine disturbances as the means by which the supposed toxins may exert their influence is also mentioned by several other writers. Pappenheim (314) voiced the conception that the worm and its toxins do not directly poison the blood, thereby producing anemia, but that they act indirectly as a toxin on certain unknown endocrine organs which have the supposed function of regulating the metabolism of fat, and which, by their dysfunction, bring about a toxic condition of the blood, spleen and bone marrow, which in turn would result in the development of anemia. Schauman (386), in a more recent paper, took up the whole question of anemia and endocrine dysfunction.

Basal metabolic deviations from the normal have not been demonstrated (32). But there are other factors which Schauman (386) thought to be of significance. The preservation of a good nutritional state reminded him of states of endocrine disturbance in which comparatively great subcutaneous fatty deposits are observed. Cessation of the menstrual periods during the attack of anemia and the relationship of pregnancy and the puerperium to pernicious anemia are suggestive in this respect. Biermer (46) and others saw in pregnancy and the puerperium, factors of etiologic significance in pernicious anemia. Naegeli (297) emphasized the curability of pernicious anemia in which these etiologic factors were operative; some patients remained free of recurrence for fifteen to eighteen years. Schauman and Saltzman voiced the supposition that also in pernicious anemia of pregnancy and the puerperium toxic substances were circulating in the blood, and Benda thought that the development of pernicious anemia depended largely on absence of physiologic hypercholesterolemia during pregnancy, which acts as a protective mechanism for the erythrocytes against hemolytic toxins originating in the ovum.

In a few instances Diphyllobothrium anemia has been recorded in association with pregnancy. A. Meyer (272) reported a case of a Russian-Polish woman, aged twenty-five years, who in the seventh month of her first pregnancy noticed the initial signs of anemia, pallor, weakness and edema. The course was progressive, the pallor took on an icteric tinge, and the temperature was more than 101°F. Six months after onset of the condition erythrocytes numbered 660,000, the concentration of hemoglobin was 10 per cent, and the patient had all symptoms, hematologically and clinically, of pernicious anemia. The first diagnosis made was of "pernicious anemia of pregnancy;" this diagnosis was changed later to "Diphyllobothrium anemia" on the finding of ova in the stools. Complete cure resulted following administration of a vermifuge which resulted in disappearance of ova, although only a few segments of the worm were expelled. Another case was reported by Shimanskiy; it occurred in a Finnish woman, aged thirty years, who during her first pregnancy and following the birth of a premature baby was bedridden on account of weakness, dizziness, fainting spells, palpitation, extreme pallor and elevated temperature. She had had gastro-intestinal disturbance, with diarrhea and vomiting, for five years prior to this illness; she became rapidly worse, was delirious for four days, and then began to improve slowly and gradually. Etiologic consideration had been given to: (*a*) pregnancy, (*b*) chronic diarrhea, (*c*) poor hygienic conditions and inadequate food, and (*d*) immigration to Russia with change of milieu. Then the patient passed segments of fish tapeworm and after a vermifuge had been given, large pieces of the worm were recovered. Acceleration in her recovery resulted, and the diagnosis was changed to "Diphyllobothrium anemia." The following year, the patient had her second pregnancy and puerperium, which was entirely normal in every respect. Two years later, she had recurrence of diarrhea and tenesmus, but tapeworms and anemia were absent. Schauman (381) cited five instances (his cases 7, 12, 30, 46, 55) in which the onset of the anemia seemed to have been activated by pregnancy or puerperium. This represents 19.23 per cent of his twenty-six female patients.

Certain similarities exist between pernicious anemia and Addison's disease (386). In certain instances of the latter there is an unexpectedly good nutritional state. Of sixty-four cases of pernicious anemia in nine there was definite pigmentation of the skin. In two of these nine cases the pigmentation extended to the oral mucosa. There is a possibility of functional insufficiency of the suprarenal glands in these cases. Roos noted indefinite anatomico-pathologic changes in the suprarenal glands in cases of pernicious anemia and Diphyllobothrium anemia. Another possibility is that pernicious anemia in itself exerts an influence on the function of the suprarenal glands. Stephan proposed as the underlying cause, hypofunctioning suprarenal cortex, with resultant disturbance of correlation of all endocrine organs. The hormonal deficiency should then bring about increased activity of the reticulo-endothelial system, and thus increased destruction of the blood. Against this effect on the blood, the hypothetically insufficient bone marrow reacts with a return to the embryonal mode of regeneration of blood.

In pernicious anemia and in Addison's disease there may be almost identical gastro-intestinal disturbances (386). Achylia is common in Addison's disease, according to Bittdorf, and the blood pressure in both conditions is usually lowered. Originally, Addison's disease was called specific anemia, and in most instances it is associated with a certain degree of anemia; even a combination of the two seems to have been observed, although extremely seldom, as both diseases are comparatively rare. Schauman (386) observed one family three members of which had respectively pernicious anemia, Diphyllobothrium anemia, and Addison's disease; in another family, one member had Diphyllobothrium anemia and another member, Addison's disease associated with severe anemia.

Simonin also considered the relation of parasitic intoxication to the endocrine system. He did not state the nature of the "worm toxin," but admitted that it might be a product resulting from regular secretions by the parasite, from the activity of its exchanges, or from modifications in the metabolism of its host, brought on by its presence. He mentioned a number of endocrine disturbances which might arise from helminthiasis. Some appeared to be concerned with metabolism; that is, emaciation had been observed in cases in which the intake of food was greater than the subject would normally require, whereas in others there had been observed deposition of fat that was attributed to retardation of processes of oxidation. Other endocrine disturbances were concerned with inhibition of growth eventuating in infantilism, loss of masculine characteristics in boys, or retarded puberty; still others, with disturbance of ovarian function, giving rise to dysmenorrhea, amenorrhoea, metrorrhagia or miscarriage, and finally some with functional insufficiency of the suprarenal glands leading to a condition of shock. Experimentally he demonstrated in various endocrine organs following acute intoxication pathologic changes, such as congestion, hemorrhages, degeneration and necrosis. Following chronic intoxication the changes he observed were hypertrophy of the pituitary gland, the thyroid gland and the suprarenal glands and degeneration of testes and ovaries.

In a number of cases, polyuria is noted and in some instances of Diphyllobothrium anemia this keeps on for some time after improvement has taken place. Elevation of body temperature is sometimes seen in conditions caused by endocrine disturbances. The cyclic course of the disease, as a whole, has parallels in disorders of the organs of internal secretion. Interstitial cirrhotic processes in the gastric and intestinal mucosa, the tongue, kidneys, pancreas and lymph nodes in cases of cryptogenetic pernicious anemia and Diphyllobothrium anemia have been interpreted as an expression of a tendency to increased formation of connective tissue, possibly due to disturbed internal secretion. Schottmüller feels that treatment with liver substitutes for, or liberates, a supposedly depressed hormone necessary in the metabolism of the blood.

It is known that the blood possesses some detoxifying properties. The so-called hemotoxins have to be administered in rapidly increasing doses in order to maintain the hematologic changes (411); if the same dose is given repeatedly, the initial changes obtained will disappear rapidly; the organism has found time and means to protect itself. Most of the extracts of tapeworms and of other organic substances with which experimenters have succeeded in producing anemia by intravenous injections into animals have been derivatives of protein (31, 308, 403). It is, therefore, in reality the effect of inoculation of foreign protein that is being studied in these animals.

The similarity between the blood picture of these experimental animals and that in cases of Diphyllobothrium anemia as well as in cryptogenetic pernicious anemia, and furthermore the contention that toxins circulate in the blood of all carriers of *Diphyllobothrium latum* led Schauman (384) to consider the possibility of anaphylatic phenomena as the underlying mechanism in the development of Diphyllobothrium anemia, granted the presence of an individual hypersensitivity toward the supposed toxin. Cederberg (83–85) elaborated on this hypothesis. He pointed out that pernicious anemia (and Diphyllobothrium anemia) has four features in common with reactions to foreign protein: (*a*) periodicity, (*b*) fever, (*c*) fluctuations in nitrogen metabolism, and (*d*) gastro-intestinal disturbances. Normally the gastro-intestinal wall affords satisfactory protection against invasion into the blood by foreign proteins. A constitutionally inferior intestine, however, may become inefficient. It may be unable to withstand constant normal or pathologically increased strain. Provided higher derivatives of protein (albumoses, peptones) should enter the blood stream in small amounts, but continually, then a condition would arise which would be similar to that in animals rendered anemic by parenteral administration of extracts of worm. Cederberg expressed the belief that the rôle of the worm is entirely secondary and nonspecific; that in some way it adds injury to the inherently weak intestinal wall. He entertained the conception that in the course of the "anaphylactogenic proteotoxicosis" a toxic principle may be liberated from the antigen-antibody-complement complex.

In the final analysis, then, the realization is unavoidable that in Diphyllobothrium anemia, as in cryptogenetic pernicious anemia, the basic etiologic factor is unknown (74, 96, 114). It must be bound to the individual afflicted and accordingly is endogenous (382, 383, 389). The question arises whether and how, except for the anemia, the anemic carriers may be distinguished from the carriers who resist the development of anemia. Whatever evidence there may be for such distinction is, to be sure, speculative and circumstantial.

Individual predisposition to a reaction which expresses itself in the development of Diphyllobothrium anemia is vigorously proposed and elaborated on by Schauman (381, 386), and his pupils, Rosenqvist (354), Saltzman (372), Lindström (242), and others. A number of other investigators, such as Schapiro, Courmont and André, Tonnel, Queckenstedt, Herzog, Cramer and Grünmandel, agree with them in their contension. Whereas Cramer expressed the opinion that such a predisposition may be acquired, over a period of time, through privation and poor hygienic conditions, he also maintained that the predisposition may rest on a congenital basis.

Schauman (382), in 1900, emphasized the individual reaction to the supposed toxic invasion:

> Ordinarily the host is refractory toward the active agent of the tapeworm, but under certain circumstances this resistance or immunity may be affected to a greater or less degree. This curtailment of defense may possibly be occasioned by varying debilitating factors, dehydration, infectious diseases, bad hygienic conditions, mental upsets, or, maybe, mostly by elements so subtile they elude our observation.

The chief requirement for the development of anemia, after all, however, must be seen in an individual constitutional predisposition. When a carrier remains free of anemia the mechanism which prevents anemia from developing may be either relative immunity of the blood or compensatory hyperactivity of the hematopoietic organs. If these functions should become insufficient, anemia would result. Schauman (383, 386) expressed the belief that the susceptibility to anemia is inseparable from the constitutional make-up of the person and also that it is inheritable. The evidence he has presented in support of this contention has been alluded to previously, and the features which seem to have a bearing on the question will be only briefly summarized here: recurrent attacks of anemia in the same person; chlorosis or secondary anemia in the history of persons in whom Diphyllobothrium anemia develops later; death from cryptogenetic pernicious anemia after previous full recovery from Diphyllobothrium anemia; familial occurrence of Diphyllobothrium anemia; occurrence of Diphyllobothrium anemia in one member of a family and of cryptogenetic pernicious anemia in another member of the same family; families with stigmas of degeneration, such as alcoholism, subnormal or abnormal mentality and morality, and extraordinary susceptibility to tuberculosis. The unknown factor in the pathogenesis of Diphyllobothrium anemia seems to be identical with that in cryptogenetic pernicious anemia. This throws light on the fact that a person who has once gone through an attack of Diphyllobothrium anemia, later may succumb to cryptogenetic pernicious anemia, "for how could this be explained without the presumption that the individual in question was equipped with a peculiar predisposition for pernicious anemia, a predisposition which could be transformed into disease under the influence of *Diphyllobothrium latum*, or some other exogenous factor, or perhaps even without the presence of any palpable exogenous factor" (382).

Queckenstedt expressed the thought that this constitutional aberration may be on the order of a specific chemical make-up of the organism, rendering it particularly susceptible to certain hemotoxins. Vlayeff expressed the feeling that the age of the host is important. He stated that infestation of long duration in a young person, who is growing, is a combination of circumstances favorable for the development of anemia.

The worm in its capacity of preciptating agent may, after all, not contain or produce a hemotoxic substance. Perhaps the chemical complexities of the body, because of the presence or influence of the worm, suffers alterations which come close to intoxication (117, 386, 389).

In animals which have been rendered anemic by experimental methods, individual variations have been observed in their reactions to the same hemotoxin, given in the same dose and with the same technic (308, 386, 404). Nyfeldt divided his rabbits into three groups: (*a*) those with strong general susceptibility to the toxin, which will have a fatal effect in doses harmless to others; (*b*) those with erythrocytes of especially high susceptibility for the toxin, whereas the other structures seem to possess marked resistance to it, and (*c*) those with very high general resistance, both of organs and of erythrocytes. Group (*b*) is, according to Nyfeldt, the one in which a blood picture typical of pernicious anemia can be produced, whereas in group (*c*) only slight transitory anemia will develop. This interesting fact furnished Nyfeldt with a plausible explanation of the failure of some investigators to repeat Seyderhelm's experiments (for example Becker (27) and Moses and Warschauer) and with an explanation of why, in some animals, typical hyperchromatic, megalocytic, hemolytic anemia develops, whereas in others, only a simple, hypochromatic, microcytic, secondary type of anemia develops, even when the technic and the toxin used are the same. It also opens up the discussion of unclassified types of anemia, such as cryptogenetic secondary anemia in man, and their relation to the primary types. It is possible that future investigations into the nature and action of the active principle in the liver and in the gastric wall may yield information which will lead to better understanding of the etiology and pathogenesis of Diphyllobothrium anemia.

Type of anemia

Cornell, in his monograph, referred to Combe as author in 1822, of the first report of a case of pernicious anemia. To Addison belongs the credit of being the first to describe, in 1855, this entity as a generic disease and to label it "idiopathic." Biermer, in 1868 gave a preliminary report, followed in 1872 by the publication of reports of fifteen cases of what he called "progressive pernicious" anemia. These and later observers sought in vain to establish a plausible etiology for this mysterious malady, although Biermer suggested the possibility that poor hygiene, the puerperium and gastro-intestinal disturbances are factors which favor its development.

Before the etiologic significance of the fish tapeworm had been realized, many cases of "pernicious anemia" which occurred in the Baltic provinces evidently had been of the Diphyllobothrium variety. This was explicitly stated by Hoffmann, Reyher and Runeberg (364). After recognition of the importance of the worm began to gain ground little hesitancy was exhibited in accepting Diphyllobothrium anemia as an example of typical "pernicious anemia" in the sense of Addison and Biermer. Early writers who held that Diphyllobothrium anemia is pernicious anemia were Botkin (55), Reyher, Mitzkuner, Runeberg (384–386), Lichtheim, Litten (245), Schapiro, Kissel, Mueller, Westphalen (470–471), Dehio (99, 101), Winiarski, Minnich, Eckert (109), Wiltschur, Askanazy, Biruli, and Vlayeff. Their contention met strong opposition from other men, such as von Holst (182), Unverricht, Biermer (47), Quincke, Immermann, and Planchard; none of these, however, had observed a case of Diphyllobothrium anemia.

Schauman (381), in 1894, dealt with material of seventy-two cases. By means of the facilities available at that time he proved conclusively that Diphyllobothrium anemia did not differ in any essential way from the type of disorder generally described as cryptogenetic, idiopathic or essential pernicious anemia. At this early stage, he emphasized the fact that the parasitism alone could not be given the whole responsibility for the development of the anemia. He assumed the existence of predisposing factors, and considered sex, age, life, occupation, physique, previous health, and heredity in this connection. The great preponderance of cases occurring in Finland and along the inner Baltic Sea drew his attention to the peculiar geographic distribution and possible racial predisposition. His observations have been the chief source of information for this treatise.

Since the appearance of Schauman's splendid monograph, very little opposition has been found to his views, particularly in Europe. Unfamiliarity with Schauman's work may account for some of the little opposition that his views have encountered.

Warfvinge, in 1900, reported a case of Diphyllobothrium anemia in which he saw only the coincidence of genuine pernicious anemia and intestinal parasitism. He contended that a causal relationship did not exist between the two conditions, since in his case, immediate recovery did not follow elimination of the worm, whereas improvement began on the tenth day of hospitalization, under the influence of arsenical medication. Ceconi doubted the identity of the anemia. Distinct clinical differences between the two types of anemia were seen by Herzog who contended that: (*a*) whereas achylia is constantly present in cryptogenetic pernicious anemia, in his case of Diphyllobothrium anemia the gastric acidity was slightly higher than normal: (*b*) whereas glossitis is present in the majority of the cases of cryptogenetic pernicious anemia, his patient gave no history or objective evidence of such a condition, and (*c*) there is a difference in predisposition for the two types of anemia. Rosenow (351, 352) who personally observed about thirty cases which could not be distinguished from cryptogenetic pernicious anemia clinically or hematologically, maintained that because of the prognostic difference between the two, it was wrong to identify them with each other. Grawitz (159) at first was willing to include in the group of "true" pernicious anemia those cases of Diphyllobothrium anemia in which the patients died in spite of expulsion of the parasite, whereas he would designate the cases in which the patients recovered, simple, secondary anemia. Later (160), however, he adopted fully Schauman's view. Matsumoto, in his report of a case of Diphyllobothrium anemia in a Japanese, stated that the anemia in his case differed from essental pernicious anemia in four respects; that is, in his case fever and hemorrhagic diathesis were absent, the blood smears were not characteristic of pernicious anemia and the patient recovered rapidly after expulsion of the worm.

In the United States and Canada the opinion is generally in favor of considering the two types of anemia apart as two distinct entities. Edsall (114) maintained that certain changes in the blood in Diphyllobothrium anemia deviated from those in pernicious anemia; he emphasized especially the lower color index and the leukocytosis without relative lymphocytosis in Diphyllobothrium anemia. He admitted, however, that these differences were not always sufficiently marked or constant to make distinction possible on the basis of the blood picture alone. Savitz stated that absence of megaloblasts and absence of leukopenia were sufficient to rule out pernicious anemia in his case. Schmidt was unable to find any instance in the literature of a case of Diphyllobothrium anemia in which the anemia exactly resembled pernicious anemia, and the morphology of the blood in his case was not typical of pernicious anemia. Isaacs, Sturgis and Smith also mentioned that many cases reported were not typical, although the diagnosis in their case indisputably was pernicious anemia. Classification of Diphyllobothrium anemia as being simply a form of secondary anemia was made by A. Meyer (272), Martin, Rosenthal, Warthin (464).

Cameron and Foster, whereas the hematologic similarity of Diphyllobothrium anemia and pernicious anemia was admitted by Martin, Warthin, Cabot (74, 75), Evans and Cornell. Excerpts which illustrate the generally accepted viewpoint read as follows: "Where *Dibothriocephalus latus* is found (in a patient suffering from pernicious anemia), it may be considered the cause of the anemia, which is not a true pernicious anemia" (94). "The presence even of a wholly characteristic blood picture does not constitute the disease, and indeed, when another condition is found to account for the anemia, pernicious anemia also is probably not present" (126). Giffin and Bowler expressed the belief that absence of subacute combined sclerosis in the cases reported, and the rapid return to normal health following expulsion of the worm spoke against their being accepted as true pernicious anemia, and they gave the timely advice that further detailed study, directed toward the solution of the problem as to whether or not Diphyllobothrium anemia and pernicious anemia are identical, be made by those who observed such cases.

In agreement with Schauman and his pupils is a formidable array of authorities, such as Lazarus, Grawitz (160), Hirschfeld, Naegeli (297, 298), Pappenheim and Vogel, besides observers of cases such as Bruhn-Fåhraeus, Neubecker, Jawein, Bard, Chemolosoff, Courmont and André, Orlowsky, Krantz, Ragosa, Cramer, Willson (475) and Thompson. Several writers have stated that they have observed cases of Diphyllobothrium anemia, indistinguishable from cryptogenetic pernicious anemia (181, 197, 351), and many others have preferred not to express any opinion as to whether or not the two forms of anemia are identical.

A few years ago Schauman was asked to contribute the chapter on pernicious anemia to Schittenhelm's handbook "Diseases of the Blood and the Blood Forming Organs." The work had not been quite finished when he died and the completion of it was left to Saltzman, who carried out Schauman's plan. This chapter, which appeared in 1925 (389), added facts to those previously reported, and emphasized again the identity of Diphyllobothrium anemia with cryptogenetic pernicious anemia, but also stressed a few points of divergence between the two types. These points are interesting in themselves, although not essential as far as diagnostic significance is concerned. These points

TABLE 22

Points of deviation between cryptogenetic pernicious anemia and Diphyllobothrium anemia

	CRYPTOGENETIC PERNICIOUS ANEMIA	DIPHYLLOBOTHRIUM ANEMIA
Tendency to morbidity (in Finland)	Increasing	Declining
Precipitating etiologic factor	Entirely unknown	Partially known
Age	Higher frequency in age group 10 to 29 years	Lower frequency in age group 10 to 29 years
Onset	Usually somewhat more chronic	Usually somewhat more acute
Course	Usually more chronic	Usually more acute
Remissions	More frequent	Less frequent
Achlorhydria	In almost 100 per cent	In 83.7 per cent, hypochlorhydria in the majority of the remainder, influenced by age
Subacute combined sclerosis and peripheral neuritis	Rather common	Not thoroughly investigated; milder symptoms rather common, marked symptoms very rare
Fragility test	Slightly more marked increase in width of resistance	Slightly less marked increase in width of resistance
Leukopenia	Somewhat more marked	Somewhat less marked
Response of reticulocytes to treatment with liver	Slightly more pronounced	Slightly less pronounced
Gastro-intestinal mucosa	Comparatively more pathologic change (quantitatively)	Comparatively less pathologic change (quantitatively)
Prognosis on proper anthelmintic treatment before introduction of treatment with liver	Mortality practically 100 per cent (deaths in hospital 46.5 per cent, Schauman and Saltzman). Not influenced by age	Mortality about 12.7 per cent, influenced by age
Response to treatment with liver	Less often refractory	More often refractory

of deviation, which have been considered earlier in this study, are listed in table 22.

It is readily seen that a consideration of anemia hardly can be profitable without definitions of the subjects concerned. Clinicians seem fairly sharply divided into two opposing groups regarding the definition of pernicious anemia.

One group is inclined to isolate the cryptogenetic type and look on it as a generic entity apart from forms of anemia which have the closest relationship to it. This group, the members of which generally admit the great clinical, hematologic and pathologic similarity between the cryptogenetic type and the types in which an etiologic factor apparently is present, is insistent on two criteria for a diagnosis of pernicious anemia; namely, the absence of any demonstrable cause and the presence of an extremely unfavorable prognosis.

The fallacy of this viewpoint is amply demonstrated by Minot and Murphy's recent introduction of liver in the treatment of pernicious anemia. This important advance changed the prognostic outlook overnight. An optimistic attitude toward medical science and its future progress would justify a sound scepticism as to the validity of the view that pernicious anemia is cryptogenetic in origin, and idiopathic in nature.

Another group of writers considers pernicious anemia to be a syndrome with clinical, hematologic, and pathologic aspects, which may be produced, or rather ushered in, by various agents, known or unknown. To me, this viewpoint seems more rational and fruitful. Cabot (74) expressed the idea as follows:

> Primary anemia is that in which the causal factors are either entirely unknown or are apparently insufficient to produce so severe a disease. . . . "Primary" means not the absence of any cause of anemia in the history, but the absence of any sufficient cause so far as is known. . . . . Given the unknown cause that does lead to "primary" anemia, and it might be that a pregnancy, a nervous shock, or the presence of intestinal parasites would act as the straw that breaks the camel's back; but the important causal factor is the unknown factor.

Keeping well in mind that there exists a form of pernicious anemia of cryptogenetic origin, according to present knowledge, and in which until recently the prognosis was hopeless, I prefer to adhere to the definition of Cabot and the other authorities belonging to the second group, which may be stated in this way: Pernicious anemia is a syndrome with a characteristic blood picture, typical pathologico-anatomic features, and fairly typical clinical manifestations, which is ushered in by some known or unknown etiologic factor.

Adoption of this definition would facilitate a unified conception of the various types of pernicious anemia, would offer a more promising basis for further investigation into the etiology and pathogenesis of these types of anemia, and would give a better explanation of various important factors involved.

It is well known that organs and groups of organs react similarly toward various etiologic agents. A certain syndrome may be considered more the expression of the efforts exercised by the organs to combat the injurious influence than the direct effect produced by the specific agent. One would hardly insist on excluding a case of cryptogenetic pleurisy from the form of disease known as pleurisy because of the one distinguishing factor, the unknown etiology. Hemiplegia is hemiplegia, whether it is caused by hemorrhage, thrombosis, embolus, tumor, abscess, or syphilitic changes in the brain, whether or not one is able definitely to determine the cause in a given case.

As far as the hematologic reaction goes in pernicious anemia, the more recent view is that there is failure of maturation and release of blood cells in the bone marrow, an altered mode of regeneration of these cells, and a certain amount of destruction of blood as a secondary feature. It is supposed that this reaction is effected by some toxin which has an inhibiting action on the hematopoietic organs and a destructive action in the intravascular spaces. This conception fits equally well Diphyllobothrium anemia.

PART IV. SUMMARY AND CONCLUSIONS

A thorough review of the literature which has a bearing on the subject of Diphyllobothrium anemia has been undertaken. Interesting and unforeseen information has been gathered and recorded. Views and opinions which are not generally known have been analyzed and formulated.

The following résumé may be given as the essence of the study: The great majority of persons who harbor the fish tapeworm in their intestines suffer no ill effects from this parasitism. In some cases there will be a variety of clinical manifestations without anemia. The symptoms may be referable to the central nervous system in the form of neurotic states, other functional disturbances or "fits;" to the alimentary tract, usually in the form of functional disorders, irregularities of the bowel, or, rather significantly, stomatitis, or glossitis; or finally to the blood, which may present slight changes suggestive of incipient anemia. The altered blood picture in nonanemic carriers has been defined as an abortive form of Diphyllobothrium anemia. Tabulation of these cases shows a gradual transition from an entirely normal condition of the blood to a state of definite anemia. The line between a nonanemic and an anemic blood picture had been arbitrarily drawn at a level for erythrocytes of 4,000,000 for each cubic millimeter.

With very few exceptions, Diphyllobothrium anemia runs true to type. As a rule it is indistinguishable from cryptogenetic pernicious anemia, clinically, hematologically and pathologically. A few points of deviation between the two types have been noted. Although none of these is of diagnostic significance, some of them deserve special mention.

Curves representing cases in hospitals in Helsingfors, from 1898 to 1927, show a steady decline in Diphyllobothrium anemia, in contradistinction to cryptogenetic pernicious anemia, which, since 1910, is rapidly increasing. The declining curve in Diphyllobothrium anemia may find an explanation in earlier recognition of the cause and earlier administration of treatment directed against the cause by family physicians, abolishing the necessity of hospitalization. The rising curve in cryptogenetic pernicious anemia, so characteristic for many countries, may be partly accounted for by early diagnosis and more extensive hospitalization, although it seems unlikely that the rise is purely relative.

Curves representing the age distribution, expressed in indexes relative to the distribution of the population in the various age groups, reveal a greater accumulation of cases in the earlier decades in Diphyllobothrium anemia. The occurrence of cryptogenetic pernicious anemia seems to be more definitely influenced by age, in the absence of any known etiologic factor. In Diphyllobothrium anemia the imposition of a notoriously deleterious influence seems capable of bringing on the anemia earlier in life.

Achlorhydria is present in about 84 per cent of cases of Diphyllobothrium anemia. When hydrochloric acid is present, the acidity is usually below the average level. Follow-up studies have shown a return of gastric acidity in more than a third of the cases in which there is achlorhydria. There is a definite influence of age on this particular feature; preservation of gastric acidity is greatest before the age of twenty and gradually diminishes with increasing age.

Subacute combined sclerosis has not been thoroughly investigated in association with the anemia under consideration. Numbness and tingling in the hands and feet are not uncommon complaints; the vibratory sense is in one instance referred to; gross lesions of the spinal cord seem to be extremely rare. It should be mentioned, however, that the frequency of extensive pathologic changes in the spinal cord in cryptogenetic pernicious anemia also has been reported variously by authors in different countries. Schauman observed only three cases in which there was evidence of gross changes in the spinal cord out of a total of 520 cases of pernicious anemia, 326 of which were cases of Diphyllobothrium anemia and 194 of which were of the cryptogenetic variety. He did not state the distribution of these three cases in the two types of anemia. One of the three patients also had syphilis.

Before the etiologic significance of the tapeworm had been duly recognized the same grave prognosis prevailed in Diphyllobothrium anemia, as existed for cryptogenetic pernicious anemia until the introduction of treatment with liver. Systematic anthelmintic measures, which were adopted about 1885, reduced the mortality from nearly 100 per cent to about 12 per cent. The cure is remarkably permanent. The hospital mortality in cases of Diphyllobothrium anemia shows a definite increase with age, from 6.8 per cent in the age groups under thirty years to 31.0 per cent after sixty, whereas in cryptogenetic pernicious anemia there is about the same hospital mortality throughout.

The etiology, which in cryptogenetic pernicious anemia is entirely unknown, is at least partly known in Diphyllobothrium anemia; that is, a definite precipitating etiologic factor is well recognized and can be eliminated.

One is easily led to the assumption that simultaneous occurrence of a tapeworm and pernicious anemia are incidental and that the presence of the parasite is of no etiologic significance, when the following facts are considered. About 750,000 persons in Finland are estimated to be infested with the fish tapeworm. Definite anemia develops in only one of 5000 to 10,000 carriers. This anemia is evidently identical with cryptogenetic pernicious anemia. Some patients die from the anemia despite removal of the worm, or after recovery succumb to pernicious anemia later, in the absence of any worm. In some instances persons who have once been cured of Diphyllobothrium anemia may later again harbor the parasite without the development of anemia.

There is, however, strong evidence of causal relationship between the parasitism and pernicious anemia. This is shown by the dramatic change which usually follows the expulsion of the worm, the rapid clinical and hematologic improvement, accompanied by a typical reticulocytic response, and the permanent cure.

It is obvious, on the other hand, that the worm cannot be the sole factor involved in the production of the anemia. Other factors of equal or greater significance must play a part. These factors are not known and consideration of them would embrace also the hidden etiology of cryptogenetic pernicious anemia. It is assumed that they have to do with peculiar constitutional properties, a specific predisposition for the development of anemia of the pernicious type.

An analysis of constitutional features incurs a study of race, family and individual. It is striking to find much higher morbidity from pernicious anemia in Finland than in Germany; also, although Finland and Sweden have a comparable frequency of the cryptogenetic type of anemia, Finland easily leads when the variety that accompanies tapeworm is added. The latter is almost unknown in Sweden.

A tabulation of about 550 cases of Diphyllobothrium anemia reported in the literature reveals the most interesting fact that more than 70 per cent of them occurred in Finlanders; Sweden, Italy, Roumania and Japan, also heavily infested, are surprisingly free of this complication. A racial predisposition seems to exist there. It might be mentioned, in passing, that the Finlanders belong to the Finno-Ugric race, entirely unrelated to the Germanic and Latin races and to the inhabitants of Roumania and Japan.

Occurrence of Diphyllobothrium anemia in two or more members of the same family has been recorded in fifteen instances. Occurrence of this anemia in one member and cryptogenetic pernicious anemia in another member has been observed in four families. This indicates a certain familial tendency.

Individual predisposition is evidenced in recurrent anemia following reinfestation, which has been observed in four or five cases. All these patients were cured also the second time. There are seventeen cases recorded, in which the patient at one time recovered from an attack of tapeworm anemia, only to succumb at a later date to cryptogenetic pernicious anemia in the absence of the parasite.

It is readily seen that the rôle of the fish tapeworm is only that of a precipitating factor, a trigger mechanism. Other intestinal parasites are similarly capable of ushering in pernicious anemia. No conclusive proof has been offered to support the claim that the worms produce or contain a specific hemotoxin. The quantity or quality of the worm or the duration of the infestation seems to be of little significance. The cases considered here have only this one thing in common: the presence of the parasite in the intestines.

Contemporary theories entertain the conception that pernicious anemia is an end-result of chronic intoxication, the intestinal contents perhaps being the source of the hypothetic toxins. In Diphyllobothrium anemia the pathogenic mechanism may be one of several possibilities. These possibilities may be broadly divided into those which have to do with the action of the parasite and those which have to do with the reaction of the host.

The worm may influence the intestinal content by giving rise to significant alterations in the bacterial flora or by rendering it toxic through interference with normal digestive processes. The worm also may influence the intestinal wall by directly or indirectly injuring it, thereby effecting increased permeability for intestinal toxins that are present. It may act as an irritant, either disturbing directly a hypothetic endocrine function of the wall, or disrupt indirectly, by way of sympathetic reflex, the harmony of interrelationships among various endocrine functions which presumably control the equilibrium of the blood. Entering the blood stream, the toxins may act directly on the blood and blood-forming organs, or they may cause alterations to arise in metabolic processes or in the biochemical milieu so as to create a situation closely resembling toxicosis. The toxins may be on the order of higher derivatives of protein.

The reaction of the host may involve failure of "detoxification." Detoxification is normally accomplished readily. The success of treatment with liver and gastric wall may indicate these organs to be the site of the detoxifying function. Incomplete detoxification seems to take place in nonanemic carriers who show clinical manifestations. Failure of detoxification apparently paves the way for the development of pernicious anemia. There is some evidence, both clinically and experimentally, favoring the hypothesis of pernicious anemia being on the order of a "chronic foreign protein reaction."

The impression is gained that there are some persons who have constitutional strength sufficient to withstand ordinary strain and the cumulative onslaught of the passing years, but who lack adequate resisting power when a new factor of etiologic significance is superimposed. These persons ordinarily can maintain biologic equilibrium, at a more or less safe level, but under conditions and at times, when circumstances seem unfavorable, the tapeworm may seem the straw that breaks the camel's back. On the other hand, the removal of the worm in such cases will, as a rule, reëstablish the status quo and disclose a favorable prognosis.

In accordance with this view it seems appropriate to classify surviving patients with Diphyllobothrium anemia as suffering from abortive forms of genuine pernicious anemia.

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A REVIEW OF EXPERIMENTAL STUDIES OF NON-SPECIFIC INHIBITION OF ANAPHYLACTIC SHOCK

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This review is limited to studies of non-specific inhibition of the anaphylactic shock which is obtained by the second injection of an antigen. Similar studies of the inhibition of anaphylatoxin, peptone and other shocks, of which there is a small but interesting bibliography, have been omitted.

The discussion of so extensive a subject as this, one which contains so many conflicting findings or statements of findings, must necessarily be broad. Detailed information will be found in the addenda, where the agents have been listed alphabetically, in order to facilitate orientation. There, under each agent, an effort has been made to review every publication critically and, where several articles have been published on the use of one agent, a brief summary has been made. We believe that the addenda will be of interest mainly to readers who wish to use it in reference to particular substances. An idea of the types of agents used for non-specific inhibition of anaphylactic shock, however, may be obtained from the following list, which includes all that we have been able to find in available literature.

Alphabetical List of the Agents Which Have Been Used to Inhibit Anaphylactic Shock

Acetone
Adrenalectomy
Adrenalin
Agar
Ageing of antigen
Albumoses
Alcohol
Allocaine
Almond oil
Ammonium sulphate
Anaphylatoxin
Animal charcoal
Antitrypsin
"Arsenobenzene"
Atropine
Barium chloride
Barium sulphate
Barometric pressure reduction
Bayer "205"
Bayer "1910"
Benzoic acid
Benzol
Bile
Blood volume increase
Brain surgery
Butyric acid
Caffein
Calcium acetate
Calcium chloride

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Calcium lactate
Calcium sulphate
Cascosan
Castration
Chloral hydrate
Chloralose
Chloroform
Cholesterin
Cholin hydrochloride
Complement fixation
Congo red
Curarin
Dextrin
Dialysis of antigen
Diastase
Dichlorathylsulphid
Drying of antigen
Egg fat
Emulsin
Eserine
Ether
Ethyl chloride
Filtration
Formaldehyde
Freezing antigen
Gastric juice
Gelatin
Gland extracts, sexual
Glucose
Glycogen
Guanidine chloride
Heating antigen
Heparin
Hirudin
Hydrochloric acid
Hydrogen peroxide
Ink
Intercurrent infection
Invertin
Iodbenzoic acid
Iodine
Iron, state of hydrosol
Iron oxide
Iron oxide, saccharated
Kephalin
Lanolin
Lecithin
Lipoids, not listed elsewhere
Magnesium sulphate
Manganese chloride
Mercurochrome-220
Mineral waters
Milk
Morphine
Mud
Multiple sensitization
Myrosin
Neoarsphenamine
Nephrectomy
"Novoprotein"
Olive oil
Opium
Orange juice
Oxygen
Pancreatin
Paraldehyde
Pepsine
Peptone
Pilocarpine hydrochloride
Pituitrin
Potassium chlorate
Potassium oxalate
Potassium permanganate
Pregnancy
Pressure
Quinine
Rice, polished
Roentgen ray
Saponin
Seed extracts
Serum fractions
Serum globulin
Serum, heterologous
Serum, normal
Sodium bicarbonate
Sodium carbonate
Sodium chloride
Sodium citrate
Sodium cyanide
Sodium formaldehyde-sulphoxylate
Sodium glycholate
Sodium hydroxide
Sodium hyposulphite
Sodium iodoxybenzoate
Sodium nitrite
Sodium oleate
Sodium persulphate
Sodium sulphate
Sodium taurocholate
Splenectomy
Starvation
Stovain
Strychnine
Succinic acid peroxide
Sulphur, colloidal
Sulphur, protein combination
Surgical interference
Talliamine
Temperature reduction
Thorium X
Thymectomy
Thyroidectomy
Thyroid extract
Toxins
Transfusion
Trikresol
Trypan blue
Ultra violet light
Urethane
Urine
Vagus section
Vitamin A deficiency
Vitamin B deficiency
Vitamin C
Water
Whey protein

Before discussing the types of experimentation employed, certain points in regard to methods of study should be emphasized. Owing to the individual variations of the experimental animals, it is absolutely necessary to use a sufficiently large number of both control and treated animals to allow a determination of the extremes of such variations. Closely related to this is the necessity of knowing, granted that a given agent inhibits shock, that the second injection of antigen has been sufficiently large; or, granted that a given agent fails to inhibit shock, that the second injection of antigen has not been excessive. This would seem quite obvious, and would not have been mentioned, had we not been forced to read article after article in which one or two controls and treated animals were offered as conclusive evidence, or in which no titration of antigen was given.

Since it is desirable not to employ an antigen dosage increased to the point of killing the least sensitive animal, which would be much more than a lethal dose for the average, one may not expect 100 per cent of the controls to die. Certainly they should all have very definite shock symptoms, especially the striking temperature drop characteristic of sub-acute shock. On the other hand, not all of the treated animals need survive, but a statistically significant proportion of them should show definitely less shock than the controls. It also seems probable that in testing agents of possible clinical application the more delicate methods of skin tests, or Friedberger's method of measuring temperature changes would be more important than work with lethal or multiple lethal doses of antigen. There might conceivably be quantitative differences, as previously indicated, which this more crude method would mask. It is also possible that many agents totally unable to inhibit shock with lethal doses of antigen would be of definite value in protecting against a degree of shock comparable to that found clinically. Of the agents studied to date, those which have shown some evidence of inhibitory action might be worthless under other conditions, and those agents which have seemed to exert no protective action might be shown to be of definite value when tested by more delicate methods.

In summarizing the findings for discussion, we have placed the experiments in different categories, as follows:

I. Those in which efforts have been made to modify the antigen to be used for shock.
  A. In vitro treatment of the antigen.
  B. In vivo treatment of the antigen.
II. Those in which efforts have been made to influence the reacting substances or tissues of the experimental animal.

A. Treatment before sensitization.
B. Treatment during sensitization.
    1. Treatment early in the sensitization period.
    2. Treatment throughout the sensitization period.
    3. Treatment late in the sensitization period.
C. Treatment simultaneously with the second injection of antigen.
D. Treatment after the second injection of antigen.
E. Treatment both before and during sensitization.
F. Treatment both during sensitization and after the second injection of antigen.

I A. THE IN VITRO TREATMENT OF THE ANTIGEN

A large number of chemical and physical agents have been used in an effort to change the antigen used for the shocking dose, but, in general, there has been very little work done on any one method of inhibition.

MATERIAL AND PHYSICAL MEANS USED IN VITRO TO MODIFY THE ANTIGEN TO BE USED FOR SHOCK

Chemical

Adrenalin
Alcohol
Ammonium sulphate
Animal charcoal
Butyric acid
Calcium acetate
Cholesterol
Eserine
Ether
Gelatin
Glucose
Gastric juice
Hydrochloric acid
Hydrogen peroxide
Iodine
Peptone
Pilocarpin
Potassium permanganate
Sodium citrate
Sodium formaldehyde sulphoxylate
Sodium hyposulphite
Succinic acid peroxide
Trikresol
Urethane

Physical means

Ageing
Dialysis
Drying
Filtration
Freezing
Heating
Ultra violet light
X-ray

Enzymes

Pancreatin
Pepsin

Glucose, gelatin, peptone, cholesterol, ether, enzymes and a number of miscellaneous substances grouped under "Chemicals," have been mixed in various ways with the antigen. Where a clear solution has resulted the mixture has been used for the second dose of antigen and where a precipitate has been formed, the filtrate has been injected. Needless to say, an assorted group of alleged antigens has been injected. Much of this work was done by Anderson and Rosenau many years ago in their fundamental studies of anaphylaxis. Their shocking dose was almost always given intraperitoneally in from 3 to 6 cc. amounts. This renders the evaluation of their negative results difficult as in other series they were able to shock sensitized animals with as little as 0.1 cc. of antigen given intraperitoneally. The antigen used by Rosenau and Anderson was usually horse serum. They mixed antigen with varying amounts of alcohol, butyric acid, calcium citrate, hydrogen peroxide, iodine, potassium permanganate, sodium citrate, succinic acid or trikresol. Of these mixtures the results from the injection of the alcoholic and hydrogen peroxide filtrates into a very small number of guinea pigs (2 or 3) demonstrated fairly good inhibition of shock. The fact that the other mixtures have negative results within the limits of the experiment is not evidence that they are without value under other circumstances. The amounts of filtrate or of mixture used were greatly in excess of the amount of untreated antigen needed to shock the controls.

Within the limits of the small number of animals used, there is evidence that Carpani, also Surányi and Jarno, working with cholesterol mixtures, Brodin and Huchet using sodium formaldehydesulphoxylate, a drug closely related to novarsenobenzol, and Von Dungern and Hirschfeld, using iodine, were able to inhibit shock.

Pancreatin and pepsin were used by Lesné and Dreyfus, who stated that they were able to obtain inhibition, but gave no protocols.

Using excised muscle strips, Larsen and his co-workers were able to prevent contraction by immersing the sensitized tissue in from 8 to 10 per cent peptone solutions before the addition of the antigen.

No inhibition was obtained by other chemical agents. It is interesting to speculate upon the changes produced in the antigen by some of the substances used. Some, such as glucose, probably act only as diluents. Others precipitate the proteins and so modify them that it is not surprising that no shock is obtained. This in no way represents desensitization and should not be accepted as such unless a subsequent injection of untreated antigen is without marked effect or causes definitely less shock than in the controls. Where the chemical employed unites with the antigen to form complex sustances quite different from the original antigen, shock obtained with such modifications may be due to something of the nature of an anaphylatoxin. In some cases it is impossible to tell whether the action of the agent is upon the antigen or upon the sensitized animal. Furthermore in cases where the antigen has been precipitated, some quantitative comparison should be made by sensitizing fresh animals with the modified antigen before like doses of the modified and unmodified antigens are assumed to be equal.

Experimental work on the various changes produced by physical means, such as ageing, dialysis, drying, filtration, freezing, heating, x-ray and ultra violet radiation is of interest. There is strong evidence that heating to 55° to 60°C. for an hour will markedly reduce the toxicity of a serum antigen. No conclusive work has been done to demonstrate any great change in the antigen by other physical agents. However the effect of ultra violet light may be significant and experiments with it should be repeated. The interesting work of Giaume would have been more valuable had he shown that his animals which had no shock when given serum irradiated with ultra violet light were then not susceptible to being shocked by the untreated antigen. Without this test he has shown no inhibition of specific shock.

It is quite possible that ageing, dialysis, drying, filtration and heating simply reduce the toxicity of the antigen. Therefore any inhibitory results obtained should be recognized as partial specific desensitization.

I B. IN VIVO MODIFICATION OF THE SHOCKING DOSE OF ANTIGEN

Most interesting experiments have been done in which a drug was given intravenously to the animal which was later bled to obtain the shocking dose of serum. With both arsenobenzene and ink some evidence has been given that the serum so treated in vivo is less toxic than the untreated serum. Again it seems to us that the proof of inhibition of specific shock would have been the subsequent injection of unmodified antigen. Further experiments along this line, however, using many different agents, might be of the greatest interest, offering as they do innumerable possibilities of variations in time and drug dosage. Such experiments more closely approximate the conditions under which the agents would normally be employed than do in vitro tests, and serve to indicate whether or not such inhibitory action might be expected in vivo, although they as yet throw no light on how drugs might be expected to act. It is obvious that the ability or inability of a drug to modify in this way the toxicity of an antigen is only half of the story, but it would be of value to know whether or not the action of certain drugs was upon the antigen or upon the sensitized animal. This method of experimentation offers the possibility of determining part of the answer to this question.

II A. TREATMENT OF THE EXPERIMENTAL ANIMAL BEFORE SENSITIZATION

With the exception of some x-ray radiation all the treatment in this division falls under the head of operative surgery.

Adrenalectomy, thymectomy and splenectomy have been performed on unsensitized animals by various workers. The removal of the organs in no way affected the later sensitization and shock of the animal.

Lanzenberg and Képinow and later others have shown quite conclusively that thyroidectomy on the unsensitized animal will in a rather marked degree lessen his ability either to develop sensitization or to react to a second injection of antigen. Control animals were all shocked.

Von Heinrich gave an erythema dose to guinea pigs on the day of sensitization. Twenty-one days later they survived the shocking dose while the two controls died. It is too bad that this original work was not better controlled. He also carried out an interesting experiment in passive sensitization. Two guinea pigs were irradiated three weeks after sensitization. Their serum was transferred to normal animals. They had no shock when given amounts of antigen fatal for two controls.

II B, 1, 2 AND 3. TREATMENT DURING THE PERIOD OF SENSITIZATION

In this section three sub-groups have been made, some of which overlap unavoidably.

Materials or Physical Means Used to Modify the Reacting Substances or Tissues of the Animal During or After the Sensitizing Period

Surgical	*Colloids*	*Proteins*
Brain surgery	Agar	Albumose
Nephrectomy	Congo red	Anaphylatoxin
Splenectomy	Kephalin	Cacosan
Surgical interference	Dextrin	Heparin
Thymectomy	India ink	Hirudin
Thyroidectomy	Saccharated iron oxide	Novoprotein
Vagotomy	Thorium	Peptone

Seed extracts
Serum fractions
Serum heterologus
Normal serum
Whey proteins

Chemical

Atropin
Allocaine
Barium chloride
Bayer 205
Benzoic acid
Benzol
Calcium chloride
Calcium lactate
Cholin hydrochloride
Curarin
Mustard gas
Guanadine hydrochloride
Iodobenzoic acid
Iron oxide
Magnesium sulphate
Manganese chloride
Mineral waters
Neoarsphenamine
Potassium chlorate
Potassium oxalate
Potassium permanganate
Quinine
Sodium bicarbonate
Sodium carbonate
Sodium chloride
Sodium citrate
Sodium cyanide
Sodium glycocholate
Sodium hydroxide
Sodium persulphate
Sodium sulphate
Sodium taurocholate

Enzymes

Pancreatin
Pepsin

Carbohydrates

Glycogen

Narcotics

Choral hydrate
Chloralose
Ether
Ether chloride
Morphine
Opium
Paraldehyde

Fats

Almond oil
Antitrypsin pepsin
Egg fat
Cholesterol
Lanolin
Lecithin
Saponine
Sodium oleate

Miscellaneous

Complement fixation
Intercurrent infection
Pregnancy
X-ray
Starvation
Transfusion
Urine
Thyroid extract
Venoms
Toxins
Vitamin A
Vitamin B deficiency
Vitamin C

Surgery. Considerable work has been done with the idea of removing the organ or organs supposedly the site of sensitization. Of the surgical procedures employed after the first injection of antigen, there may be cited operations on the brain, nephrectomy, splenectomy, thymectomy, thyroidectomy, vagotomy and a miscellaneous group of operations, chiefly abdominal, which have been listed under "Surgical Interference." With the exception of operations on the brain, the results of these surgical procedures have been definitely negative or inconclusive. There is some evidence that trephining and incising the dura will lessen the degree of shock. Among the operations listed under "Surgical Interference," hepatectomy is the only one that seems to have any tendency to reduce shock. However, when one considers the state of man or animal after undergoing this ordeal the procedure is one of purely academic interest.

The work done on vagus section suffers from lack of sufficient numbers of experimental animals. There is, however, some evidence that bilateral or unilateral vagotomy diminishes the degree of shock. In view of the rather definite results obtained by atropinization it seems only logical to expect some definite protection. More work should be done in this aspect of the problem.

For one reason or another the other types of operations were ineffectual in inhibiting shock. As in other experiments of this general nature, one encounters much work that cannot be interpreted on account of poor controls, small series of animals, lack of proper antigen titration, etc.

Colloids. Agar, Congo red, dextrin, India ink, kephalin, saccharated iron oxide and thorium-X are the agents included in this section. Agar, Congo red and thorium X gave negative results. The experiments made with India ink and saccharated iron oxide are particularly interesting. Definite modification of shock has been obtained with the latter. The work done with India ink is not entirely conclusive, but should be repeated. Moldovan and Zolog reported an original observation which, if corroborated, is important. Serum withdrawn from non-sensitized animals two and seven days after intravenous injection of India Ink, seemed to protect sensitized animals to whom it was given five minutes before the shocking dose.

Careful work has been done with dextrin and kephalin, but it would be fallacious to believe that any definite inhibitory effect has been shown.

Proteins. One would naturally suppose that the non-specific proteins as a group would contain the greatest number of anti-anaphylactic agents and the most effective. A study of List V which contains the names of the agents for which there is conclusive evidence of inhibitory action, does not warrant this assumption. On the other hand there is incomplete but possible evidence that further investigation with this type of agent will yield more complete proof. Heparin has been tried extensively by a number of workers and there seems to be no doubt of its protective action.

Albuminose in the experiment cited was injected three days before the shocking dose. This is evidence in neither direction. The results of the work with hirudin and novoprotein are quite inconclusive.

A number of other proteins have been given during the period of sensitization or just before the shocking dose. Anaphylatoxin, cascosan, peptone, seed extracts, serum fractions (albumin, globulin, euglobulin), normal sera, and whey protein comprise the important ones. All of these materials have shown some ability to decrease or inhibit the amount of shock in the sensitized animal. However for reasons such as lack of control animals, failure to titrate the effective shocking dose of antigen, paucity of experimental animals, varying dosages spread over a series of animals in groups of one or two, and others, sufficient positive or negative evidence has not been adduced.

There has been an enormous amount of work done upon the possible inhibitory effects of serum fractions, and of whole heterologous and homologous sera. In each group there is evidence which points toward the inhibitory effect of these agents. However, in each group there is an occasional well controlled experiment which shows no inhibition. Also there is, we regret to say, a great deal of worthless work. It is important that conclusive experiments be carried out in this field.

Cascosan has been but little used, and the conclusions in regard to it need corroboration. Peptone has caused much discussion as an inhibitor of shock. There are well controlled investigations on both sides of the question. Whey protein may very possibly inhibit shock but the statement cannot be made from the small number of animals tested.

Enzymes. No work worthy of consideration has been done. Pancreatin and pepsin have been used on only one or two animals.

Carbohydrates. In spite of the careful work on glycogen the results obtained were inconclusive.

Narcotics. Under this heading as well as under others, much work has been reported which must be taken on faith or on authority. General statements and broad assertions are made that such an agent does or does not inhibit shock. There is, however, no mention of details.

The investigations with chloral hydrate have been for the most part well conducted and present definite evidence of inhibition of shock.

Chlorolose, morphine, opium, and paraldehyde, have been reported very incompletely. No conclusion can be gained from the reports.

There has long been a belief, mainly, we fear, founded on authority, that ether as a narcotic will inhibit shock. Besredka first made the statement. A few other investigations yielded affirmatory or contradictory findings. Strangely enough we can only find one investigator who has thought it advisable to present his protocols. His results are inconclusive.

We cannot find that inhalations of chloroform have been used as an anti-anaphylactic agent.

Fats. Many of the substances of this sort which have been studied, have been used with the idea of lowering surface tension and thereby inhibiting shock.

Antitrypsin has been used by Jobling and Petersen. They have brought evidence which they considered to indicate that the acetone insoluble part of egg yolk is similar to antitrypsin. In a very small number of control and experimental animals they were able to show that the amount of shock was diminished by injection of antitrypsin just before the shocking dose.

Cholesterol and lecithin have been investigated at length, but not sufficiently to demonstrate clearly the inhibitory effects of either of these agents. Most investigators believe that they have some evidence of inhibition but none of them considers the matter settled.

Lanolin may or may not be efficacious as we could only read the abstract taken from a Japanese journal. A general statement is made about saponin. Sodium oleate would seem to be a very suitable agent to use. The work done inclines one to believe that inhibition may be obtained if the proper sequence of dosage and time be worked out.

Chemicals. Among this mass of agents, salts, acids, bases and one oil, we have found seven substances by means of which the investigators have been definitely able to inhibit shock within the limits of the experiment. Barium chloride, barium sulphate, benzol, certain mineral waters, sodium bicarbonate, sodium sulphate and sodium chloride, have all been used; mineral waters and sodium chloride have been studied quite extensively. In order to protect the animal by the latter it must be given in such large doses that sickness and death frequently result from the therapeusis.

The investigations of guanidine and neoarsphenamine have been good but corroboration is needed before definite proof of inhibition is to be accepted.

On the negative side Bayer 205, benzoic acid, curarin, iodobenzoic acid, magnesium sulphate and mustard gas have been conclusively shown not to inhibit sensitization within the limits of the experiment.

As one might expect, there are certain substances which increase the susceptibility of the animal to shock. Quinine, sodium citrate, sodium cyanide and sodium persulphate all appear to have this ability.

A number of other agents have been tried in a careless manner which makes the results worthless. Iron oxide, potassium chlorate, potassium oleate, potassium permanganate, sodium hydroxide and sodium sulphate have been used in this way.

Articles have been published about cholin hydrochloride and the bile salts which state that some inhibition has been obtained but unfortunately insufficient details were included so that this statement should be corroborated.

Miscellaneous. In this section belong some interesting experiments. The idea of exhausting complement in vivo by the intraperitoneal injection of an hemolytic system occurred to Loeffler. When this was done one hour before the shocking dose was administered the two treated animals lived and the two controls died. Perhaps this phenomenon may be explained upon the same basis as the observation that infecting sensitized animals at various times during the period of sensitization brings about de-sensitization. By this action it is also possible markedly to decrease or inhibit the reaction to the second injection of antigen. On the other hand, by varying the dosages or the infecting agent, other workers have been able to increase the sensitivity of the animal.

Several investigators have published reports which tend to show that gravid guinea pigs lose their sensitization. This seems to return at the termination of pregnancy.

Although as stated before, x-ray exposure before the initial injection seems to decrease the sensitivity of the animal, exposure during the period of incubation has no such effect.

The effects of starvation and of increasing or decreasing the vitamin content of the food are interesting. Animals kept without food for several days before the administration of the shocking dose seem to be less sensitive. Unfortunately there is no conclusive work on this subject. This type of investigation naturally falls somewhat in the class of the vitamin deficiencies but other factors may enter. The factor of starvation was somewhat controlled by feeding sensitized rats for two weeks on a diet deficient in vitamin A. The animals did not become more sensitive. However it seems that the lack of vitamin B may cause an increased sensitization. This was brought about by a diet of polished rice. The lack of vitamin C, like vitamin A, did not cause an increase in sensitization. In one experiment the intraperitoneal injection of sterile orange juice throughout the period of sensitization caused an inhibition of shock. This result is so similar to that produced by injections of the mineral waters that it is fallacious to draw any conclusions as to the effectiveness of vitamin C as an anti-anaphylactic agent.

A special type of transfusion which may or may not fall under the head of non-specific protein was carried out by almost exsanguinating a sensitized dog. Following the injection of 1200 cc. of blood from a normal dog, the recipient still reacted to the shocking dose of antigen.

The use of intraperitoneal injections of urine from anaphylactic guinea pigs into sensitized animals may also be similar in principle to the investigations on mineral waters. The animals so treated were definitely protected. No work was done with normal urine or with protein free or dialysed urine.

The work on venoms and toxins is not conclusive, but we might expect the same results with proteins in general.

II C. TREATMENT SIMULTANEOUSLY WITH THE SECOND INJECTION OF ANTIGEN

The anti-anaphylactic effect of administration of substances simultaneously with the second injection of antigen is almost identical with that obtained by administering the agents toward the end of the period of sensitization. A few of the agents were given in this manner only; most of them were used in both groups.

Agents Given with the Second Injection of Antigen

Physical
Barometric pressure
X-ray
Cooling

Narcotics
Chloroform
Ether
Novocain

Chemicals
Atropin
Barium chloride
Barium sulphate
Bayer 205
Manganese chloride
Oxygen
Pilocarpin
Sodium nitrite
Stovain
Talliamine

Gland or body extracts
Adrenalin

Almond oil injected just before the second dose of antigen has been used with inconclusive results.

The amount of work done with atropin is large. It is described under this heading as the best results, i.e., in preventing shock, are obtained when it is given just before the second injection of antigen. Well controlled investigations have given results which satisfactorily demonstrate the ability of this drug to inhibit shock.

The investigations with chloride or sulphate of barium, with Bayer 205 and with narcotics have been described. Those with manganese chloride, sodium nitrite, talliamine, adrenalin and pilocarpin are inconclusive.

Oxygen inhalations and stovain intravenously seem to give some inhibition; but the subject needs further study.

The physical methods used such as lowering of barometric pressure and chilling protected a certain number of the animals, x-ray radiation was given without effect.

II D. TREATMENT AFTER THE SECOND INJECTION OF ANTIGEN

It is very difficult to evaluate the investigations falling within this group. In the first place little has been done; adrenalin and urethane have been used in combination; potassium permanganate was tested on only one animal; talliamine was tested on a small number and may have prolonged life. The work on calcium chloride is equally inconclusive. Some of these substances may give protection, but none has been definitely demonstrated.

II E. TREATMENT BEFORE AND DURING SENSITIZATION

In this group are included x-ray, trypan blue and starvation, none of which gave definite protection.

SUMMARY OF AGENTS INHIBITING ANAPHYLAXIS

After analysis of the protocols of the studies reported in the addenda, we have summarized those agents for which conclusive evidence of the inhibition of anaplylactic shock has been presented. Our criteria have included the number of controls, the number of treated animals, and the titration of the antigen. We have not included in this list agents which have been reported without careful protocols. This does not mean that such work is necessarily unsound, but without such protocols it has been impossible to make accurate analyses of the experiments. With these restrictions in mind we have collected from the records in the addenda the agents for which there is conclusive evidence of inhibitory action. These are presented in the following list:

Agents for Which There is Conclusive Evidence of Inhibitory Action

1. Atropine, given before the second injection of antigen.
2. Barium chloride, given before, or added to, the second dose of antigen.
3. Barium sulphate, given after the second injection of antigen.
4. Reduction of barometric pressure, after the second injection of antigen.
5. Benzol in large doses, before and during sensitization.
6. Trephination before the second injection of antigen.
7. Calcium chloride with and after the second injection of antigen. (Clinical use.)
8. Chloral hydrate before the second injection of antigen.
9. Heating of the serum antigen.
10. Heparin before the second injection of antigen.
11. Intercurrent infection with tuberculosis.
12. Saccharated iron oxide during the sensitization period.
13. Mercurochrome-220.
14. Milk (aolan).
15. Certain mineral waters during the sensitization period.
16. Multiple sensitization and treatment with one of the several antigens.
17. Sodium bicarbonate before the second injection of antigen.
18. Sodium carbonate before the second injection of antigen.
19. Sodium chloride before the second injection of antigen.
20. Sodium formaldehyde sulphoxylate, added to the second dose of antigen.

At this time there is too little knowledge of the exact method of action of this diversified group of 18 agents to allow conclusions of any value to be drawn from them. It should also be remembered that their efficiency can be recognized only within the definite limits of the investigative work.

There are a number of agents concerning which there is incomplete but possible evidence of inhibitory action. These agents are presented in the following list:

Agents for Which There Is Incomplete but Possible Evidence of Inhibitory Action

1. Adrenalin, before and after the second injection of antigen.
2. Alcohol treatment of the serum antigen.
3. Allocaine before the second injection of antigen.
4. Ammonium sulphate precipitation of the serum antigen.

5. Anaphylatoxin, given before the second injection of antigen.
6. Antitrypsin, given before the second injection of antigen.
7. Arsenobenzene, injected intravenously into animals later bled to supply the second dose of antigen.
8. Chloroform, given intravenously before the second injection of antigen.
9. Cholesterin, before or added to the second dose of serum antigen.
10. Cholin Hydrochloride before the second dose of antigen.
11. Complement fixation, before the second dose of antigen.
12. Egg fat, before the second dose of antigen.
13. Ether, given intravenously before the second dose of antigen.
14. Hydrogen peroxide treatment of the antigen.
15. Ink, during sensitization, or given animals later bled to supply the second dose of antigen.
16. Iodinization of the serum antigen.
17. Kephalin, before the second injection of antigen.
18. Lecithin, before the second injection of antigen.
19. Manganese chloride, before the second injection of antigen.
20. Neoarsphenamine, in passive anaphylaxis.
21. Oxygen, before the second injection of antigen.
22. Peptone, before the second injection of antigen.
23. Pilocarpine hydrochloride, before or with the second dose of antigen.
24. Pregnancy.
25. Roentgen ray.
26. Seed extracts.
27. Serum fractions.
28. Serum globulin.
29. Serum, normal.
30. Sodium hyposulphite with the second dose of antigen.
31. Sodium iodoxybenzoate, before the second injection of antigen, in skin tests.
32. Sodium oleate.
33. Starvation.
34. Temperature reduction of the sensitized animal before shock.
35. Thyroidectomy.
36. Thyroid extract in small amounts by mouth two days before shock.
37. Ultra-violet light irradiation of the serum antigen used for shock.
38. Urine from anaphylactic animals.
39. Vagotomy, before the second injection of antigen.
40. Vitamine C deficiency during sensitization.
41. Whey, given before the second injection of whole milk.

SUMMARY OF AGENTS WHICH INTENSIFY SHOCK

Among the agents that intensify shock we find a number which also may inhibit it, depending usually upon the dosage. Intercurrent infection, which apparently may either intensify or diminish shock, acts in an entirely unknown manner.

Agents for Which There Is Some Evidence of Intensifying Action

1. Adrenalectomy, before the second injection of antigen.
2. Benzol, in small doses before and during sensitization.
3. Heterophile antigen, before the second injection of antigen.
4. Intercurrent infection during sensitization, or before sensitization.
5. Quinine, before the second injection of antigen.
6. Sodium citrate, before the second injection of antigen.
7. Sodium persulphate, before the second injection of antigen.
8. Thyroid gland extract by mouth, in large amounts, before the second injection of antigen.
9. Trypan blue.
10. Vitamine B deficiency during the sensitization period.
11. Mud from Hot Springs.

THEORIES TO EXPLAIN MODE OF ACTION OF INHIBITING AGENT

The theory most in vogue at present is that of *changes of colloidal* equilibrium. Such changes, involving an increased viscosity and lowered surface tension of the blood, have been shown to have been brought about by certain mineral waters and by ether or chloroform given intravenously. It seems probable also that many of the agents studied cause such changes, although they have not been studied from this point of view.

The theory of *reticulo-endothelial block* has been advanced by various investigators working with ink, saccharated oxide of iron, lanolin and other agents. Such block is supposed to work in one of two ways, first, by plugging the reticulo-endothelial system *before* the first administration of antigen, and second, by blocking the reticulo-endothelial system of the sensitized animal and therefore preventing shock. There is, however, no authoritative evidence that these agents do inhibit shock, nor that, should they protect, that they would do so by such a blockade. Furthermore, there is no evidence that it is possible to blockade the reticulo-endothelial cell completely.

The theory of *exhaustion of antibodies by a non-specific protein* is comparable to that observed in vitro in complement fixation. Under this group may be listed intercurrent infection, the injection of heterologous sera, of peptone, of anaphylatoxins, of serum fractions, of whey protein, or sensitization with several antigens. Intercurrent infection is the only one of these agents which has been definitely proven to inhibit shock except for multiple sensitization and treatment with one of the antigens. This theory is entirely unproved and these agents may act in some other, as yet unknown way.

The theory of *neurogenic action* may be utilized to explain the diminution of anaphylactic shock in guinea pigs brought about by the use of atropine and adrenalin. There is definite knowledge that atropine will inhibit shock in guinea pigs and probably this is by its action on the vagus. Adrenalin has not been definitely shown to inhibit shock, but in the experiments recorded, the action may be interpreted as being due to the direct action of the drug on the sympathetic system. The action of chloroform and of ether as anesthetics is entirely unknown.

Action by a *modification of antibody production* is of particular interest because it may be stretched to cover almost any body reaction. Of the agents which have been found to have some such probable inhibition, 3 varieties are found.

First, such agents as benzol, which is thought by some to have a direct action on antibody production.

Second, agents generated within the body itself as a result of metabolic changes, as for example, vitamin B or C deficiency, starvation or pregnancy.

Third, agents which produce changes indirectly in antibody production, as by the removal of certain organs. Thyroidectomy has been conclusively shown to decrease markedly the ability of the animal so treated before sensitization to respond with the same degree of shock as the controls.

A number of agents have been used because they in themselves *induce shock in some way similar to anaphylactic shock.* Peptone, anaphylatoxins, urine from guinea pigs that have had anaphylactic shock, are examples of such agents. No conclusive work has been done to show that any protection obtained with such agents is due to their shocking properties.

On the other hand, a number of agents have been tried because they *produce results opposite* to those found in anaphylactic shock. Chief of these are substances which increase the blood pressure, such as barium chloride and adrenalin, drugs which relax the bronchial muscles, such as atropine or adrenalin, and agents, such as the narcotics, which affect the central nervous system. Lowering of the barometric pressure has also been used because it produces results which are, as a whole, opposed to the effects of anaphylactic shock. Starvation has been used because of its accompanying leucopenia, which was supposed to offset the leucocytosis observed in shock.

Certain other theories have been advanced of which there is even less conclusive support than for those just discussed. Among this last group are agents which *exhaust complement*, which *increase the blood volume*, as in pregnancy or the administration of sodium chloride, or *saturate the precipitable elements of the blood* as barium sulphate. The theory of modifying the specific nature of the antigen has been discussed previously. It is futile to enter into detail in regard to these theories, as not one has been proved; their very diversity signifying how utterly confused has been the entire problem.

In summary, we know of no facts or any theory which will explain conclusively the definite non-specific inhibition of anaphylactic shock that is produced by a variety of agents. It is well worth the endeavor of chemists, physicists, physiologists, immunologists and clinicians to throw further light upon this problem.

ADDENDA

Discussion of Agents, in Alphabetical Order

Acetone

Surányi and Jarno, in 1928, obtained no inhibition of shock when the serum used for the second injection had been treated with acetone. All five of the animals given treated serum died, as well as the five guinea pigs given normal serum. These experiments were controls for more successful tests with cholesterinized serum.

Adrenalectomy

Képinow, in 1922, found that upon the removal of the suprarenal tissue, guinea pigs could be killed by 0.025 cc. of serum, whereas the controls required 0.1 cc. The number of controls was not stated. This is one of the rare instances of apparent intensification of shock.

Adrenalin

Anderson and Schultz in 1909 made an exceptionally well-controlled study of the action of this drug in experimental anaphylaxis. Thirty days after intraorbital sensitization, guinea pigs were treated before the second injection of antigen, probably given intravenously. The exact interval between treatment and shock was not stated. No animals were treated with adrenalin alone, the drug being given intraperitoneally simultaneously with the intravenous administration of chloral hydrate and urethane, or with artificial respiration with oxygen, or with all three. The results may be summarized as follows:

TREATMENT	TOTAL	DIED	LIVED	
			Number	Per cent
Adrenalin, chloral hydrate and urethane	12	7	5	41
None	12	10	2	16
Adrenalin, chloral hydrate and urethane, and artificial respiration with oxygen	12	4	8	66.6
None	13	11	2	15
Artificial respiration only	16	9	7	43
None	16	13	3	18

Unfortunately, owing to the combined treatment, the exact rôle of the individual agents cannot be determined. Certainly the best results were obtained by treating with all four. In every series the difference between the treated animals and the controls was striking. This is one of the few papers in which combined treatment was given, a field which offers unlimited opportunity for further study.

In 1918 *Pelz and Jackson*, sensitized dogs with 3 injections of horse serum, or of egg white. It was found that adrenalin was of value if given early after the shocking dose of antigen, but of no value if not given until the spasm had thoroughly developed. The experiments were not comparable to those in which treatment preceded the shocking dose of antigen.

Hanzlik and Karsner in 1920, studying the action of adrenalin in shock from many different substances, reported a few experiments with serum anaphylaxis. No untreated controls were given for these particular experiments. In all, only 4 sensitized guinea pigs were treated with adrenalin, given intravenously either just before, with, or after the shocking dose of antigen. Two animals died and 2 survived after shock, with marked dyspnoea. Nevertheless, the authors concluded that "when injected together with the antigen or immediately preceding it, epinephrine in the dosage of 0.0005 cc. of 1:10,000 per gram of body weight intravenously prevents death from true anaphylactic shock in guinea pigs." For obvious reasons, such conclusions do not seem justified by the evidence presented in the paper.

Galambos, in 1913, treated serum-sensitized guinea pigs with intravenous adrenalin before the second injection of antigen by the same route. The lethal doses of antigen and of adrenalin were determined. In all, 12 animals received $\frac{1}{60}$ mgm. of adrenalin, followed by multiple lethal doses of antigen from 15 to 75 minutes later. In many instances, only one animal was used for each variation of antigen and of time interval. In general, however, there was evidence that protection might be obtained with adrenalin against 3, or rarely more, lethal doses of antigen. The authors also seemed justified in concluding that the time of treatment was important. Too few experiments were done with other dosages to warrant any conclusions, including one experiment in which both atropine and adrenalin were given.

Levy-Solal and Tzanck in 1923 failed to save either of 2 guinea pigs by adding non-lethal doses of adrenalin to the shocking dose of serum, given intracardially. Not only was the series negligibly small, but no determination of the lethal dose of antigen was made. The amount given, 1 cc., from which all of the controls died, probably represented many lethal doses. These authors cited without reference the work of Garrelon, Santenoise and Tirrel, which we have been unable to trace.

Summary. (1) No conclusive evidence is offered by Hanzlik and Karsner, nor by Levy-Solal and Tzanck that adrenalin modifies shock when combined with the second dose of antigen.

(2) Some evidence, not wholly convincing, has been presented by Galambos to show that adrenalin alone may protect when given before the shocking dose of antigen. Anderson and Schultz combined adrenalin with other treatment, so that the exact rôle of the adrenalin could not be determined.

(3) Some protective action of adrenalin, given dogs soon after the shocking dose of antigen, has been shown by Pelz and Jackson.

Agar

Besredka, in 1920, failed to inhibit shock by giving agar intravenously. This paper, which gave no protocols, is discussed under "Anaphylatoxin." It is inconclusive.

Aging of antigen

Besredka, in 1907-b, found that although the act of aging serum diminished its toxicity, some shocking effect could be obtained even with a 13-year-old sample.

Rosenau and Anderson, in 1908, however, found sera from 2 to 118 days old markedly toxic.

Although this aging of the antigen is obviously closely related to specific desensitization, it is cited here as an instance of attempted modification of the antigen.

Albumoses

Heyde, in 1912, obtained no modification of shock by treating serum-sensitized animals with albumoses 3 days before shock. Although no protocols were given, the work seems to have been adequate controlled.

Alcohol

Rosenau and Anderson, in 1906, treated 25 cc. of serum with from 5 to 10 cc. of 95 per cent alcohol. The mixture was kept at 15°C. for 40 hours and then filtered. Two sensitized guinea pigs which received 6 cc. of this filtrate intraperitoneally died, as well as the animals receiving the same amount of untreated serum. Three animals survived the injection of 3 cc. of the treated serum Other controls given 0.1 cc. of the untreated serum died, so that 3 cc. represented about 30 lethal doses.

Besredka, in 1908, stated that he obtained inhibition of shock by the narcotic effect of alcohol. No protocols were given.

Allocaine

Lumière and Enselme, in 1926, treated sensitized guinea pigs intravenously with 2.5 mgm. of allocaine in 0.5 per cent solution and found that they then resisted amounts of antigen lethal for an unstated number of controls.

Almond oil

Achard and Flandin, in 1911, obtained no inhibition of shock by giving from 5 to 20 cc. of almond oil just before shock. The experiments were not conclusive.

Ammonium sulphate

Rosenau and Anderson, in 1906, obtained questionable inhibition of shock by precipitation of the serum antigen with ammonium sulphate. Subsequent dialysis removed the sulphate and other inorganic salts. Six cubic centimeters of this filtrate injected intraperitoneally caused death in 1 guinea pig, 5 cc. killing 1 of 2. Controls were killed with 6 cc. of untreated serum. This antigen dosage was high, because the authors showed that 0.1 cc. of serum usually killed susceptible animals.

Anaphylatoxin

Lurà, in 1912, failed to inhibit shock by treating 2 sensitized guinea pigs with Bacillus prodigiosus anaphylatoxin, given intraperitoneally 24 hours before shock with 1 lethal dose of antigen. Although both untreated controls and anaphylatoxin controls were used, no conclusions can be drawn from so small a series.

Aronson, in 1912, gave sensitized guinea pigs sub-lethal doses of Bacillus prodigiosus anaphylatoxin intravenously 1 day before the second injection of the antigen. All of the treated animals lived and all of the untreated animals died. Their number was not given.

Besredka, in 1920, made a general statement that sensitized animals, treated intravenously with suspensions of agar, were not protected against a minimum lethal dose of antigen.

Dale and Kellaway, in 1923, concluded that "acquired tolerance to anaphylatoxin does not involve desensitization of the anaphylactic animal, nor does specific desensitization involve tolerance to anaphylatoxin." This seems to have been clearly shown for both passively and actively sensitized guinea pigs, when the second injection of antigen followed from 3 to 25 minutes after the administration of anaphylatoxin. However, when the shocking dose of antigen was not given until 2¼ hours after the injection of anaphylatoxin, none of the 3 animals so treated had shock. In another series anaphylatoxin immune serum failed to protect 1 animal shocked 5 minutes after treatment. But because normal serum also partially protected 1 animal shocked 70 minutes after treatment, the authors concluded that the protection obtained by the immune serum was not due to its immune qualities. It is unfortunate that in these carefully controlled and beautifully planned experiments, much larger series of test animals were not used.

Summary. In spite of the importance which this aspect of the problem would seem to have upon the relation of anaphylatoxin shock and anaphylactic shock, there is no conclusive evidence as to whether anaphylatoxin shock does or does not inhibit anaphylactic shock. Further studies along this line, with some quantitative determination of the amount of protection obtained, if any, would certainly be of interest. Protection with anaphylatoxin against anaphylactic shock, however, would not be proof of the identity of the two types of shock.

Animal charcoal

Besredka, in 1907, found that contact of the serum antigen with animal charcoal did not reduce the toxicity of the serum.

Antitrypsin

The question of changes in the antitrypsin content of serum in anaphylactic shock involves many complexities.

Seligmann, in 1912, reported investigations showing that the antitrypsin increase in shock was not marked.

Ando, in 1913, was also unable to demonstrate any specific increase of antitrypsin in anaphylactic shock, that is, the increase was no greater than after the sensitizing injection of antigen, or after the injection of an heterologous substance.

Jobling and Peterson, in 1914, further complicated the question. They showed, to their satisfaction, that antitrypsin was an acetone-soluble lipoid, which could be obtained from serum or egg yolk, and that the injection of such an extract raised the antitrypic titre of normal serum. They concluded that, by treating sensitized animals with the subcutaneous injection of antitrypsin before the second injection of antigen, guinea pigs could be made refractory to shock, because of the increase of antitrypsin in their serum. An analysis of the protocols presented makes it difficult to regard the conclusions as entirely justified, on account of the small number of animals used. Thus, in the antitrypsin series, of the 3 treated animals, 1 died and 2 lived, while both of the comparable controls died. It is possible that in a larger series, some of the controls might have lived, or more of the treated animals have died. The results with lipoid-free antigen are inconclusive for the same reason. More definite evidence is presented in the series given egg fat before shock, as all of the 5 animals treated before and after the first injection of antigen lived when shocked, while both of the controls died after receiving the smallest amount of antigen given the treated animals.

In 1919–20 there appeared the excellent study on antitrypsin by *Teale and Bach.* In showing that serum antitrypsin was pròtein in nature and not lipoidal, they disagreed with most of the work of Jobling and Peterson. Furthermore, Teale and Bach found that in acute anaphylactic shock, there was no diminution of antitrypsin, while in delayed shock, the fall was not appreciably greater than with the sensitizing injection, as had been shown by Ando. Teale and Bach did not try to desensitize with antitrypsin.

Rysznyák, in 1920, found a slight rise in the antitryptic titre of serum in anaphylactic shock during the summer months. Animals were found to be more sensitive in the winter, when the minimum lethal dose of antigen was less. No protocols were given in this brief note.

Launoy and Falque, in 1922, using 4 guinea pigs, found no change in the antitryptic titre of the blood in anaphylactic shock fatal in 4 or 5 minutes.

Summary. Considerable work has been done upon the antitryptic titre of the blood serum before and after anaphylactic shock.

1. There is no agreement as to whether antitrypsin is a protein or a lipoid.

2. There is no agreement as to whether the antitryptic titer goes up or down in anaphylactic shock.

3. The work of Ando and of Teale and Bach is well done and shows very little change after fulminating shock. A slight drop was observed after delayed shock but no more marked than in the controls.

4. Jobling and Peterson as well as Pfeiffer and Jarisch, found that there ensued a fall in antitryptic titre after anaphylactic shock.

5. Rusnyák found that the amount of antitrypsin varies with the season and is less in the winter when sensitizing ability is greater. The converse of this is true.

6. The only connection between these investigations and non-specific desensitization is the work of Jobling and Peterson. This simply shows that there is some acetone soluble substance in egg-yolk which when injected into animals before the shocking dose may protect them from death. Consequently their work should also be considered under the heading of lipoids.

Arsenobenzene

Suarez and Schaefer, in 1928-b, tried a unique *in vivo* method of modifying the serum used for shock Rabbits were given 0.5 gram of arsenobenzene intravenously 8 hours before they were bled to obtain the serum which was used to shock guinea pigs previously sensitized to normal rabbit serum. It was found that the minimum lethal dose of the treated serum for guinea pigs previously sensitized with rabbit serum was 1.75 cc., while for the untreated serum controls it was only 0.33 cc. Parallel experiments with hen serum showed that while the untreated serum was lethal for sensitized guinea pigs in a dose of 0.04 cc., if the serum was obtained after treatment with arsenobenzene, the lethal dose was 0.25 cc. for unheated serum, or 0.5 cc. for heated serum. For the action of another arsenical, see Neoarsphenamine.

Atropine

Auer and P. A. Lewis were among the first to try the effect of atropine in experimental anaphylaxis. In their preliminary report in 1909, they suggested that the intravenous injection of 0.5 to 1 mgm. of atropine just before the shocking dose of antigen would decrease or prevent the fatal pulmonary signs of shock. In the *Journal of Physiology* in 1910, *Auer* published his first protocols. His work was divided into several sets of experiments in most of which 1 or 2 treated animals were compared with a similar number of controls, that is, no allowance was made for individual variations in the animals. In one set, however, there were 11 controls, 9 of which died and 2 of which recovered from moderate or severe shock while of the 8 animals given 3 mgm. of atropine subcutaneously, only 1 died and 7 had mild or no shock. This seems to be an entirely valid experiment in favor of atropine. In summarizing his experiments, Auer found that of the animals tested within a month after sensitization, 13 of the 17 controls died, while only 4 of the 17 treated animals succumbed to shock. In a small group of animals, held about 224 days after sensitization, no significant difference was found between the controls and the treated animals.

Mita, in 1911, objected to Auer's work on the ground that not all of his controls died. This criticism is, in our opinion, invalid. Mita used routinely 2 mgm. of atropine sulphate, given intravenously 15 seconds before shock. The amount of antigen used to shock was calculated per 100 grams of body weight, which does not appear necessary. Mita, testing animals sensitized with pigeon, swine or beef serum could find no significant variation in favor of the treated animals.

Auer in 1912, replying to Mita, believed that the latter's failure to obtain protection was due to the insufficient dosages of drug used.

The early work of *Anderson and Schulz* in 1909, with atropine, failed to show any marked difference between the 9 untreated control guinea pigs, 2 of which lived, and the 14 animals treated intraperitoneally with 0.01 mgm. per gram of atropine before shock. Only 4 of the treated animals lived. However, large doses of antigen were used for shock and given intraperitoneally.

Biedl and Kraus, in 1910, working with dogs, stated that atropine lessened anaphylactic shock. No protocols were given.

Friedberger, in 1911, criticised Auer for the same reason that Mita did, and cited experiments by Mita and himself, in which the amount of the shocking dose of antigen was sufficient to kill all of the controls. He failed, however, to consider that his shocking dose of antigen may have been too high to allow the measuring of differences which would have been appreciable by more delicate methods. Two milligrams of atropine, given intravenously, failed to protect any of 6 guinea pigs from the lethal effects of 0.1 cc. of horse serum antigen per 100 grams of body weight. In another set of animals, sensitized to swine serum and given a shocking dose of antigen of 0.3 cc. per 100 grams of body weight, 1 of the 6 treated animals lived.

Karsner and Nutt, in 1911, approached the problem by determining a constant minimum lethal dose of shocking serum. The dosage of atropine was raised until the minimum protective dose was found. The shocking dose of serum was then increased and the minimum amount of drug necessary to protect was redetermined. This method was continued up to the toxic limits of atropine. Both drug and antigen were given intravenously.

2 mgm. of atropine protected 1 animal against 0.4 cc. of serum.
2 mgm. of atropine protected 1 animal against 0.6 cc. of serum.
7 mgm. of atropine protected 1 animal against 0.8 cc. of serum.
25 mgm. of atropine protected 1 animal against 1.0 cc. of serum.

Protocols were not given for the controls, but the results of Karsner and Nutt were sufficiently definite to be of great value.

Galambos in 1913, gave 1 animal for each variation different amounts of atropine at different times before multiple lethal doses of shocking antigen. Although controlled, the experiments are difficult to evaluate and in our opinion the author has little justification for his conclusions that atropine does protect, and that the time of treatment and dosage are important.

Pelz and Jackson, in 1918, working with dogs, found that atropine was of no value if given after the shocking dose of antigen.

In *1920 Hanzlik and Karsner* treated two serum-sensitized guinea pigs with atropine, 3 and 5 mgms. No controls were given, but the authors concluded that atropine could partially protect against serum shock. This conclusion hardly seems justified from the experiments cited.

Levy-Solal and Tzanck, in 1923, in uncontrolled experiments, found that animals had slight or no shock when atropine was given with the shocking dose of antigen.

Stoland and Sherwood, in 1923, using the excised, sensitized, uterine muscle of virgin guinea pigs and 1 per cent. solutions of atropine, found that the response of the sensitized muscle to the specific antigen was prevented by atropine. Larger doses of atropine did not increase the muscle tone so much as small ones.

Zunz and LaBarre, in 1924, found that 1 mgm. of atropine per 100 grams of weight prevented anaphylactic shock if given intraperitoneally to sensitized animals 20 to 35 minutes before the intravenous injection of 1 minimum lethal dose, or slightly more, of antigen. In the treated animals that were completely protected, the surface tension and reaction of the blood remained normal. From 0.25 to 0.5 mgm. of drug per 100 grams did not protect and the surface tension and hydrogen ion concentration of the blood were diminished as in the controls. It is unfortunate that the authors did not present protocols of either controls or of the treated animals.

Summary. The work of any value which has been done that tends to show the favorable effect of atropine on anaphylaxis is small. Auer's experiments are good, but he did not repeat his only successful one. Karsner and Nutt have shown that some protection can be obtained. The reports by Biedl and Kraus and by Zunz and La Barre contain no protocols.

On the other hand, there are three well-controlled investigations by Mita, by Friedberger, and by Anderson and Shultz, which show no evidence of the effectiveness of atropine in allaying shock.

The work of Stoland and Sherwood, well done though it is, seems of questionable value inasmuch as we do not know that *in vitro* effects can be interpreted as similar to those occuring *in vivo*.

The outstanding factor in favor of the correctness of Auer's work is that he used larger doses than those who were unable to corroborate his work. To this one must add the quite conclusive work of Karsner and Nutt showing that large amounts of atropine are necessary to protect against increasing amounts of the shocking dose of antigen.

Barium chloride

Biedl and Kraus, in 1909, employed barium chloride in dogs. Although no protocols or dosages were given, the authors concluded that after the blood pressure of sensitized dogs had been raised with barium chloride, the subsequent administration of a shocking dose of antigen failed to cause the drop in blood pressure characteristic of anaphylactic shock. Even when treatment was not given until after shock, when the blood pressure had reached its lowest level, a rise of pressure could be obtained with barium chloride.

Pfeiffer and Jarisch, in 1913, working with guinea pigs, found that barium chloride gave definite protection when injected intraperitoneally in doses from 0.005 to 0.05 gram, 30 minutes before the intraperitoneal injection of the shocking dose of antigen. These experiments were well controlled. They are not strictly comparable to those of other workers, who used lethal doses of antigen, as Pfeiffer and Jarisch gave doses of antigen small enough to allow a study of temperature changes in mild shock. Other experiments in this same paper, however, are comparable, and showed that protection was obtained when 0.004 gram of barium chloride were given before the intravenous injection of the shocking dose of antigen. In other experiments, barium chloride was added to the shocking dose of antigen, in the proportion of 8.7 cc. of serum and 1.3 cc. of a 10 per cent solution of the drug. Two cubic centimeters of this mixture protected the 6 treated animals. Four controls died.

Summary. The work of Biedl and Kraus cannot be evaluated on account of the lack of details. The study of Pfeiffer and Jarisch offers valid evidence that anaphylactic shock can be inhibited with barium chloride, given either before or with the shocking dose of antigen.

Barium sulphate

Lumière and Couturier, in 1921-a showed that the intracardial injection of 0.5 cc. of 0.265 per cent emulsion of barium sulphate, given just before the intracardial injection of antigen, would protect guinea pigs against 1 minimum lethal dose of antigen. Their hypothesis was that the drug saturated the precipitable elements of the blood, so that no further precipitation could occur upon the injection of antigen. The experiments are conclusive in regard to the protection afforded, if not in regard to the explanation advanced.

Barometric pressure reduction

Lumière and Couturier, in 1923, found that shock could be attenuated or repressed if guinea pigs, after the second injection of antigen, were placed under barometric pressure reduced from 30 to 40 cm. of mercury. In the first series 17 of the 20 controls died and 3 had acute shock, that is the mortality was 85 per cent. Of the 25 animals kept under reduced pressure for 6 minutes, 8 died at once and 14 had moderately severe shock, 2 of these dying later. Excluding these 2 animals, the authors considered this a mortality rate of 32 per cent. In a second series of 160 animals, the mortality for the controls was over 80 per cent and for the treated animals under 40 per cent. The details of these experiments were not given. However, this was undoubtedly one of the largest and best controlled series of experiments done and it offers definite evidence that a reduction of barometric pressure can influence the degree of shock.

Bayer 205 (Germanin)

Makarowa and Zeiss, in 1926, stated that anaphylactic shock could be prevented in serum-sensitized animals by the addition of 2 per cent of Bayer 205 to the shocking dose of serum. We have been unable to find the protocols for this work.

Schmidt, in 1926, from an uncontrolled study of 3 guinea pigs, concluded that 0.3 gram per kilogram of Bayer 205, given subcutaneously, could protect pigs from shock.

Collier, in 1927, in experiments which included 1 control animal, decided that Bayer 205 given before or with the shocking dose of antigen prevented or inhibited shock in 50 per cent of the treated animals.

Wiechmann and Koch, in 1928, criticized Collier's work and presented adequately controlled experiments to show that 0.2 gram of Bayer 205 afforded no protection when given 1 day after the first injection of antigen, 1 day or 1 hour before the shocking dose of antigen, or combined with the latter.

Summary. The only valid work on Bayer 205 is that of Wiechmann and Koch, which shows conclusively that, under the test conditions, the drug, given before or with the second dose of antigen, confers no protection against anaphylactic shock. It would be of interest to carry out experiments with Bayer 205 similar to those of Swarez and Schaeffer with "arsenobenzene," that is, treating the shocking serum in vivo.

Bayer 1910

The work of *Collier*, in 1927, is invalid for reasons discussed above.

Benzoic acid

Amberg and Knox, in 1912, obtained no inhibition of cutaneous reactions in sensitized animals treated with this drug, as a control for experiments with sodium iodoxybenzoate.

Benzol

Schiff, in 1915, studied benzol because of its selective action on the hematopoetic system and in relation to antibody formation. Guinea pigs were treated with multiple small doses of benzol, 0.01 cc., intraperitoneally, both before and after sensitization. There was a slight average increase in the number of white blood cells. The treated animals were more sensitive than the controls. An analysis of the protocols, however, shows that the series of treated animals and of controls were small, with several variations of shocking dosage of antigen, so that the differences observed might well have been within the limits of experimental variation. But when the benzol dosage was increased to 0.03, which caused a drop in the white blood count, there was a clear cut difference in favor of the 11 treated animals, of which 9 lived. Six of the 7 controls died. The author believed that benzol did not cause an absence of shock, but rather a lowering of the sensitivity.

Bile

Doerr, in 1908, cited the use of bile, but gave no reference.

Blood volume increase

The inhibition of shock in pregnancy was considered due to an increase in blood volume by *Lumière and Couturier 1922.* Bornstein also attributed the inhibitory action of sodium chloride to the same cause.

Brain surgery

Friedberger and Gröber in 1913, noting the parallelism between anaphylaxis and poisoning with members of the morphine group found that the latter caused first a dilatation and then contraction of the brain tissue, with an irritation of the vagus center, resulting in the lung findings characteristic of morphine poisoning. Believing that trephination should remove or at least reduce the action of such poison upon the nervous system, Gröber made experiments to test this point and found that such actually was the case. The authors therefore, were interested in studying anaphylaxis in the same way. After sensitization guinea pigs were trephined and the dura widely incised. Of the 8 controls 7 died after shock with 0.23 to 0.35 of antigen per kilogram, the lungs showing characteristic changes, 1 control surviving 0.26 cc. of antigen. Of the 8 trephined animals, none died at once after shock and when death finally occurred the lungs were slightly or not at all distended. The authors therefore concluded that in most cases animals would be protected against 2 or 3 minimum lethal doses of antigen, although death sometimes occurred within ¾ of an hour, even after only one-fifth of a lethal dose.

Schürer and Strassmann in 1912 extirpated the cerebrum of 6 sensitized guinea pigs. While both controls died from 0.1 cc. of antigen (horse serum) given intravenously, of the 5 treated pigs given 0.1 cc., 1 lived 48 hours, 2 lived 24 hours, 1 died soon, its lungs showing no distension and 1 died at once. One animal given 0.2 cc. of antigen also died at once. The authors concluded that the brain extirpation did not confer protection.

Butyric acid

Rosenau and Anderson, in 1906, obtained no inhibition of shock by treating 25 cc. of the serum used for the second injection with 10 cc. of N/10 butyric acid. The mixture was kept for 40 hours at 15°C. Three cubic centimeters of the filtrate killed 1 guinea pig. Controls were killed with 6 cc. intraperitoneally. Since many controls were also killed by the intraperitoneal injection of 0.1 cc. of serum, it is impossible to evaluate this one test.

Caffein

Doerr, in 1908, cited the use of this drug, but without reference.

Calcium acetate

Anderson and Rosenau, in 1908, gave 0.1 mgm. of this drug subcutaneously to 1 guinea pig 24 hours before shock with 0.5 cc. of serum given in the brain. No protection was obtained against what may have represented many lethal doses of antigen.

Calcium chloride

Netter, in 1906, reported a carefully controlled clinical experiment upon the administration of calcium chloride to children who were given diphtheria antitoxin. In the control group of 264 cases, including 258 which had no calcium chloride and 6 insufficiently treated cases, the number of cases of urticaria was 41, an incidence of 15.53 per 100. In the group of 252 cases given 1 gram a day of calcium chloride by mouth on the day of the serum injection and for 2 days thereafter, there were 6 cases of urticaria, an incidence of 2.38 per 100. This makes a difference in incidence between the controls and the treated cases of 13.15 per 100, which is statistically significant.

In a later article, in 1926, *Netter* recommended that treatment be proportionate to the amount of serum given.

Besredka, in 1907, made the general statement that calcium chloride prevented shock if given the night before the second injection of antigen.

Rosenau and Anderson, in 1908, however, reported no inhibition of shock by giving calcium chloride in a dosage of 0.1 gram the day before shock. Only 1 animal was used. In Hygienic Laboratory Bulletin No. 50, in 1909, these same authors reported no effects with from 0.1 to 0.15 gram of calcium chloride given subcutaneously to 3 guinea pigs 10 minutes after they had received intraperitoneally 6 cc. of the shocking dose of antigen. Other experiments reported in this study, in which calcium chloride was given to counteract the effects of magnesium sulphate, were also unsuccessful.

Biedl and Kraus, in 1909, made an unsupported statement that Netter's work was without value.

Belin, in 1911, giving from 4 to 12 doses of 5 centigrams each by mouth for 2, 4 or 6 days before shock, in a total series of 3 guinea pigs, found that these 3 treated animals recovered after acute shock, while the 1 control died. The antigen was injected into the brain. No conclusions can be drawn from this experiment.

Belin, in 1913, concluded that calcium chloride never inhibited shock when given with the antigen.

Summary. The clinical experiment of Netter seems to offer valid evidence of the value of calcium chloride, but the experiments of Besredka and of Belin, also favorable, do not present acceptable proof. Rosenau and Anderson have presented definite evidence that, under their test conditions, the drug was without value when given either before or after the shocking dose of antigen. The test conditions, however may have represented many lethal doses of antigen.

Calcium lactate

Netter, in 1906, made a general statement that calcium lactate could be employed as well as calcium chloride to prevent serum sickness.

Kastle, Healey and Buckner, in 1913, studied the effect of calcium lactate upon anaphylaxis in the guinea pig. Unfortunately the results were inconclusive because of the insufficient controls, although the authors believed that good protection was obtained by treating female guinea pigs during the sensitization period.

Summary. There is no definite evidence that calcium lactate has any inhibitory effect upon anaphylactic shock.

Calcium sulphate

See Perrin and Abel, 1927, under Mineral Waters.

Cascosan

Rusznyâk and Korânyi, in 1927, studied this and other heteroproteins in guinea pigs sensitized with horse serum. The method used was that of observing temperature changes. Treatment with "Cascosan" and other proteins, caused a rise of temperature. Subsequent injection of from 0.5 to 1 mgm. of the specific antigen, given intraperitoneally, failed to cause the rise of temperature usual after the administration of such small amounts of antigen. No protocols were given and only a few temperature charts. Nevertheless, the work is of interest.

Castration

See the article by *Lumière and Couturier*, 1921-b under Pregnancy.

Chloral hydrate

Benzhaf and Famulener, in 1908, found that 75 per cent of their sensitized animals could be protected from shock if they were given chloral hydrate in doses just sufficient to produce hypnosis. The drug was given in a 10 per cent solution, 75 mgm. or 100 mgm. per gram of body weight. Injections were made intramuscularly, 20 to 30 minutes before the intraperitoneal injection of 5 cc. of horse serum, the shocking dose of antigen. Upon recovery from the drug, from 1½ to 2½ hours later, the treated animals were free from symptoms.

Banzhaf and Famulener, in 1910, obtained excellent results when the shocking dose of antigen was given intraperitoneally 20 or 30 minutes after the intramuscular injection of from 85 to 180 mgm. of chloral hydrate. Eleven of the untreated controls died and 12 of the 13 treated animals lived. Similar treatment 20 to 30 minutes before shock was of no value, however, when the shocking dose of antigen was given intracardially. When both drug and antigen were given intracardially, 8 out of 9 animals were protected by from 1 to 3 injections of drug from 2 to 4 minutes before injection of the antigen. Multiple intracardial treatments 12 to 15 minutes before shock were of no value in 2 cases. The experiments were well controlled and the paper is one of the best we have encountered.

Rosenau and Anderson, in 1909, *or Anderson and Rosenau*, 1909, found that 200 mgm. of chloral had no marked effect when given intramuscularly in a 5 per cent solution, either 2 hours or from 21 to 31 minutes before the intraperitoneal injection of the shocking dose of antigen, 6 cc. of horse serum. Three guinea pigs, given 250 mgm. of chloral 30 minutes before the same amount of antigen recovered, after having shock symptoms. Controls given for these tests are to be found in the authors' publications in 1906. From these it is found that 6 cc. represents multiple lethal doses of antigen.

Anderson and Schulz, in 1909, as previously cited under "adrenalin" obtained inhibition of shock by treating with a combination of chloral hydrate, urethane and adrenalin, also with artificial respiration with oxygen. The exact rôle of the chloral hydrate in these tests could not be determined, because no experiments were done with that drug alone. Careful drug toxicity controls were made.

Summary. Banzhaf and Famulener have offered satisfactory evidence that chloral hydrate may inhibit anaphylactic shock when given before the shocking dose of antigen and under certain conditions, not under others. The negative results of Rosenau and Anderson are less valid on account of the lack of controls, as the shocking dose of antigen in their tests may have been too high.

Chloralose

Besredka, in 1908, stated that 0.05 gram of this drug caused animals to live several hours after shock, the controls dying in 2 to 3 minutes.

Chloroform

Rosenau and Anderson, in 1908, found that the addition of 0.4 per cent of chloroform to serum for preservative purposes had no inhibitory effect upon shock.

Kopaczewski, Roffo and Roffo, in 1920, working on the theory that chloroform lowered the surface tension of the blood, tried to inhibit shock by giving chloroform intravenously in water. The injection of 1.5 cc. of a 1 per cent solution of chloroform intravenously just before the intravenous injection of 0.5 cc. of the shocking dose of antigen protected all of the animals so treated, all of the controls dying. The number of animals used was not stated. Nevertheless, the experiments were of interest.

Summary. The action of chloroform as an anesthetic has not been studied. Its presence in the shocking dose of serum in the amount usually employed for purposes of preservation has been shown by Anderson and Rosenau not to inhibit shock with large amounts of antigen. The intravenous administration of chloroform has been found by Kopaczewski, Roffo and Roffo to protect an unstated number of animals and this is the most valuable of the contributions on the use of chloroform in anaphylaxis.

Cholesterin

Achard and Flandin, in 1911, made a general statement that results with cholesterin were less certain than with ovolecithin, but details were not given.

Carpani, in 1920, injected cholesterin, 3 cc. of a 5 per cent solution in olive oil and protected sensitized guinea pigs from shock. The series was small but well controlled.

Surányi and Jarno, in 1928, sensitized guinea pigs with 1 cc. of horse serum, a very large amount in proportion to the amounts commonly used. From 22 to 25 days later, some of the animals were given cholesterinized serum and others normal serum. The shocking dose of antigen was given intracardially, the dosage not being stated. Of the 10 controls, given normal serum, 9 died, and 1 had mild shock. Of the 10 animals treated with cholesterinized serum, 1 died, 7 had bad shock with recovery and 2 had mild shock. Controls of serum treated with acetone were not protected. Giving animals daily feedings of 0.25 gram of cholesterin, mixed with their food, for about 24 days during the sensitization period had no effect.

Summary. The experiments of Surányi and Jarno were valid and showed that cholesterinized serum was less toxic than normal serum for the shocking dose. While these findings are unquestionable for the conditions of the experiment, it is possible that on account of the large amount of antigen used for sensitization, the animals were less sensitive than those used in most of the experiments by others on inhibition of anaphylactic shock. The work of Carpani, in which guinea pigs were injected with cholesterin in olive oil before shock, also showed definite protection.

Cholin hydrochloride

Zunz and LaBarre, in 1923, found that 2 centigrams of choline hydrochloride, given intraperitoneally 15 to 25 minutes before the injection of a minimum lethal dose of antigen would protect a certain number of guinea pigs from death.

Complement fixation

Loeffler, in 1910, exhausted complement in vivo by the intraperitoneal injection of a haemolytic system (3 cc. of sheep red blood cells and 1 cc. of anti-sheep serum). This was done 1 hour before the second dose of antigen. No shock occurred if the necessary amount of treatment had been given. The shocking dose of antigen was 5 cc., given intraperitoneally. While both controls died, the 2 treated animals lived. Although the series was small, the idea is of interest. A control of the amount of complement present after treatment would have been of value.

Congo red

Karsner and Ecker, in 1924, obtained no inhibition of shock with from 0.1 to 0.005 mgm. per kilogram of this colloid, given in 1 or 0.5 per cent suspension either 1 or 24 hours before 2 lethal doses of antigen. Of the 13 animals treated, 3 died from the drug, 6 of the 7 remaining died when given 2 lethal doses of antigen 1 hour after treatment. Of 3 treated animals, given 2 lethal doses of antigen 24 hours after treatment, 2 died. The number of controls was not given.

Curarin

Auer and Lewis, in 1920, found that a "liberal dose" of curarin, i.e. 1 or 2 mgm., given intravenously, caused no inhibition of shock, artificial respiration being given after the drug.

Dextrin

Karsner and Ecker, in 1924, treated sensitized guinea pigs with from 0.4 to 0.8 mgm. per gram of this colloid. Of the 12 treated animals, 2 died from the drug. Of 6 treated animals given at an unstated time a dose of antigen lethal for 2 controls, 2 died and 4 recovered from shock. Four treated animals given the same amount of shocking dose of antigen 24 hours after treatment all lived, 2 controls dying. The authors, however, taking into consideration the variations in reaction obtained in the control series, did not consider this definite inhibition of shock, a conclusion most welcome after those of many other authors. The description of these experiments with dextrin is not entirely clear.

Dialysis of the antigen

For the negative results of Anderson and Rosenau, see Ammonium Sulphate.

Diastase

Doerr, in 1908, cited the use of diastase, but without reference.

Dichlorethylsulphid (mustard gas)

Corper, Black and Moore, in 1922, gave maximum non-lethal doses of mustard gas subcutaneously before or with the sensitizing injection of antigen, or during the period of sensitization, without any effect upon the degree of shock. This trial of mustard gas was based on previous experiments by Hektoen and Corper, in 1921, in which it had been shown that the gas, as a leucotoxic agent, exerted an inhibitory effect upon the production of antibodies.

Drying of the antigen

See also Heating of the Antigen.

Rosenau and Anderson, in 1906, found that drying horse serum did not destroy its toxic properties. Only 1 animal was used and there may or may not have been a diminution of toxicity. This method of modification is closely related to specific desensitization.

Egg fat

See Antitrypsin, for the work of *Jobbling and Petersen* on egg fat.

Emulsin

Doerr, in 1908, cited the use of emulsin, but without reference.

Eserine (physostigmine)

Levy-Solal and Tzanck, in 1923, added this alkaloid to the shocking dose of antigen, given intracardially, without inhibiting shock. The experiment was not of value, as about 2 animals were used, which were given the large amount of 1 cc. of serum for shock.

Ether

I. Combined with the antigen. Besredka, in 1907, obtained no inhibition of shock by extracting the serum antigen with ether.

II. Used as an anesthetic. Besredka, in 1907, reported that if sensitized guinea pigs were anesthetized with ether before the second injection of antigen, 0.25 cc. of horse serum, given in the brain, the animals had no shock, and upon recovery were desensitized. No protocols were given. In 1908, *Besredka* stated his belief that ether acted by making the nerve cells indifferent to the antigen-antibody combination.

Rosenau and Anderson, in 1908, produced anaphylactic death in 7 of the 8 guinea pigs etherized before the injection of the shocking dose of antigen. This was 6 cc., given intraperitoneally, which according to the 1906 controls of these authors, represented many lethal doses.

Banzhaf and Famulener, in 1908, were likewise unable to inhibit shock with ether narcosis.

Thomsen, in 1917, found only insignificant differences between the controls and treated guinea pigs which varied in weight from 340 to 400 grams. The treated animals were deeply etherized before the intravenous injection of the shocking dose of serum. In a second series, in which the animals weighed from 800 to 900 grams, there was a difference in favor of the anesthetized animals. In a third series, of which no protocols were given, the weights of the animals being from 550 to 400 grams the results confirmed those of the second set. However, if more highly sensitized animals were used, they were as little influenced by narcosis as the smaller animals of the first set. The author believed that when shock was inhibited by ether narcosis, it was due to an interference with the antigen-antibody reaction, possibly by the action of the narcotic on the lipoids of the blood tissues. Unfortunately so many different doses of antigen were given to shock in these tests, that not enough evidence was available to make any given test conclusive, as no allowance was made for the individual variations of the animals.

III. The intravenous injection of ether without narcosis. Kopaczewski, Roffo and Roffo, in 1920, working on the theory that ether and other substances lowered the surface tension of serum, tried to repress shock by giving sensitized guinea pigs 2.5 cc. of an aqueous solution of ether, saturated at 15°C. The number of animals used was not given, but it was stated that all of the controls died upon the intravenous injection of 0.5 cc. of the serum antigen, while the treated animals lived. In view of these findings, the authors believed that the action of the nervous system in shock was of less importance than changes brought about by colloidal flocculation.

Summary of experiments with ether. Evidence that the ether extraction of serum antigen does not influence its ability to shock has been offered by Besredka, without protocols.

Experiments, also without protocols, by Besredka, showing that deep ether narcosis inhibited shock, have not been confirmed by Rosenau and Anderson, or by Banzhaf and Famulener. However, the shocking dose of antigen used by Rosenau and Anderson represented multiple lethal doses and Banzhaf and Famulener gave no protocols. The experiments of Thomsen, in which there was some evidence of inhibition of shock under certain conditions, are not striking, because of the diversity of tests done with a small number of animals.

The inhibition obtained by Kopaczewski, Roffo and Roffo by giving ether intravenously seems to be valid, but would be more conclusive if the number of animals used were known.

It may be said that there is no absolutely final and conclusive demonstration that ether inhibits anaphylaxis, whether given with the shocking dose of antigen, or administered before shock as a narcotic or intravenously.

Ethyl chloride

Besredka, in 1908, obtained good protection with this narcotic, but gave no details of his experiments.

Filtration

Rosenau and Anderson, in 1906, found that filtration of horse serum through a Pasteur-Chamberland filter B did not destroy the antigen's toxic properties.

Formaldehyde

Doerr, in 1908, also gave no details as to the use of formaldehyde.

Freezing and thawing of the serum antigen

Besredka, in 1919, noted that freezing and thawing did not modify the toxicity of the antigen.

Gastric juice

Lesné and Dreyfus, in 1911, obtained no inhibition of shock by adding porcine or canine gastric juice to the sensitizing or shocking dose of antigen, actinocongestin.

Gelatin

Belin, in 1911, gave an unstated number of sensitized guinea pigs 20 per cent gelatin with the shocking dose of serum antigen without obtaining any protection.

Gland extracts

For failure to inhibit shock by the administration of sexual gland extracts, please see Lumière and Couturier, under Pregnancy. See also Thyroid Extract.

Glucose

Richet, Brodin and Saint-Girons, in 1919, found in dogs that diluting the shocking dose of serum to 9 times its volume with 1 or 4.5 per cent glucose caused no inhibition of shock. The amount of antigen, however, may have been too high. More experiments should be done with glucose.

Glycogen

Karsner and Ecker, in 1924, gave 0.025 and 0.05 mgm. per gram of glycogen to sensitized guinea pigs. When treatment was given 45 minutes before shock, all of the 3 animals which had received the smaller dose of glycogen, as well as 3 of the 4 which had been given the larger dose, died in acute shock. When treatment was given 24 hours before shock, all 3 of the animals given the smaller dosage of glycogen died, but 3 of the 4 given the larger dose lived. The authors, however, believed that to conclude that there had been inhibition would be fallacious.

Guanidine chloride

Heyde, in 1912, gave guanidine to guinea pigs sensitized with beef serum. His reason for doing so was that guanidine, present in the urine of anaphylactic animals, itself produced shock. No protocols were given. Half of the controls died, but the treated animals had no shock when given antigen intraperitoneally 3 days later.

Heating of the serum antigen

Rosenau and Anderson showed that heat modified the toxicity of serum, whether undiluted (1906), diluted (1908) or dried (1909).

Besredka, in 1919, summarized his experiments with diluted serum. He both confirmed Rosenau and Anderson and showed that the toxicity of serum could be lowered by heating at 56° or 60°C. on successive days.

This work is obviously closely related to specific desensitization.

Heparin

Keyes and Strauser, in 1926, found that while all of their 12 control pigeons had "multiple shock symptoms," 11 of the 12 birds treated with 1 cc. of 1 per cent heparin 45 minutes before the second dose of antigen had no shock and the twelfth mild shock. The work was well controlled and of value.

Hyde, in 1927, found that all of his serum-sensitized control guinea pigs died when given 1 cc. of antigen intravenously. The animals given 7 mgm. of heparin 45 minutes before shock also died. Ten mgm. of heparin given 5 minutes before shock failed to protect. From these experiments he concluded that heparin did not inhibit fatal issue. This conclusion in our opinion is not valid, because no titration of the antigen was given and the amount used, 1 cc., may have represented many lethal doses.

Reed and Lamson, in 1927, obtained no inhibition of shock by giving 40 mgm. of heparin intraperitoneally from 10 to 40 minutes before the intraperitoneal or intracardial injection of the shocking dose of antigen. The sensitizing dose of antigen was high, from 2 to 5 cc. of serum. Of the 22 controls, 50 per cent died within 24 hours after shock, while of the 28 treated animals, 49 per cent. died within the same period.

Williams and van de Carr, in 1927, treated guinea pigs with heparin from 15 to 145 minutes before shock and found that of their 11 treated animals, only 1 had acute shock, while of the 8 controls, 2 lived and 6 died. They concluded, with validity, as follows: "The results obtained appear to indicate that the injection of heparin into the circulation in amounts sufficient to prevent the formation of a blood clot prevents or reduces, in the majority of cases, the symptoms of anaphylactic shock in guinea pigs hypersensitive to horse serum."

In 1928, *van de Carr, Lyons and Williams*, in a careful study, found that "heparin, even when given in amounts sufficient to render the blood incoagulable for 72 hours will not prevent or alleviate the anaphylactic syndrome in guinea pigs when the colloidal balance of the blood has been disturbed by the previous injection of heterophile antigen."

Hanzlik, Butt and Stockton, in 1928, studying the reciprocal action of the crop muscles of pigeons in anaphylactic shock got no protection with heparin given 45 to 90 minutes before the second dose of antigen. The amounts of heparin and antigen used were not given.

Summary. The experiments of Keyes and Strauser and of Williams and van de Carr showing inhibition of anaphylactic shock with heparin were carefully controlled and valid. The work of Hyde, with negative results, is less valuable because there was no titration of the shocking dose of antigen. The negative experiments of Reed and Lamson were well controlled, but the sensitizing dose of antigen had been high and most of their animals were shocked intraperitoneally. The in vitro studies of Hanzlik, Butt and Stockton are not strictly comparable to the in vivo work. More sound evidence in favor of some protection under certain circumstances is available, therefore, than is negative evidence under other conditions.

Heterophile antigen

Van de Carr, Lyons and Williams, in 1928, found that heterophile antigen, given intravenously to guinea pigs previously sensitized with casein, so altered the colloidal state of the blood as to increase the toxicity of the second injection of antigen. The neutralization of the inhibitory action of heparin by an injection of heterophile antigen has been cited under Heparin.

Hirudin

Friedberger, in 1910, stated that inhibition of blood coagulability by hirudin was without influence, even against a small multiple of the minimum lethal dose of antigen. Two controls were used. Seven animals given hirudin at different times before shock all died. The amount of the shocking dose of antigen was calculated per 100 grams of body weight. A general statement was made that hirudin had no effect if given 24 hours before the antigen.

Zunz and van Geertruyden-Bernard, in 1921, found that the minimum lethal dose of antigen was increased if the serum had been treated from 3 to 4 hours before injection with 2 mgm. of hirudin per 7 cc. of serum. If from 2 to 4 mgm. of hirudin was given intravenously to sensitized animals from 2½ to 4 hours before shock, protection was obtained, but not against many lethal doses of antigen. No details of these experiments were given.

Summary. There is no conclusive evidence that hirudin inhibits shock; the negative results of Friedberger were fully as conclusive as the evidence of Zunz and Van Geertruyden-Bernard that some protection was obtained.

Hydrochloric acid

Lesné and Dreyfus, in 1911, obtained no inhibition of shock by adding 0.33 per cent of this acid to the sensitizing or to the shocking dose of the antigen, actinocongestin.

Hydrogen peroxide

Rosenau and Anderson, in 1906, added 5 cc. of this drug to 25 cc. of serum. The mixture was kept at 15°C. for 40 hours and then filtered. Three or 6 cc. of the filtrate was injected into the peritoneal cavities of 2 sensitized guinea pigs respectively and both recovered. In 1906, these authors demonstrated that 0.1 cc. of horse serum would kill sensitized animals. If the amounts of treated and untreated serum were comparable, these 2 animals had been protected against many lethal doses of antigen.

Ink (*China*)

Michelazzi, in 1920, treated 4 sensitized guinea pigs with 2 cc. of 20 per cent China ink 48 hours before shock. Two of the animals died that were given more than the minimum lethal dose of antigen. Two survived, 1 of these had had slightly more than the lethal dose and 1 less. The controls were given a larger sensitizing dose of antigen and therefore were not comparable.

Moldovan and Zolog, in 1923, used 0.25 cc. of China ink per 100 grams of body weight, the ink being diluted with equal parts of water. They found that no protection was obtained when the ink was given intravenously either 8 minutes or 15 days before the minimum lethal dose of antigen. If the ink was given from 1 hour to 10 days before the shocking dose of antigen, animals were protected against from 2.5 to 4 minimum lethal doses, larger amounts of antigen killing. No protocols of the experiments were given.

Moldovan and Zolog, in 1923, in an article following the one just cited, explained the protective action of China ink as due to the fact that after the deposition of carbon in the endothelial cells of the liver, a substance was secreted which prevented bronchial spasm and which was antagonistic to the active principle of anaphylaxis. Pituitrin given between ink injection and shock removed protection. No protocols are given to confirm this generality.

Moldovan and Zolog, in a third paper (1925), performed some original experiments which would be of value if confirmed by a larger series. Rabbits were injected intravenously with 5 cc. of 1 per cent China ink diluted with equal parts of water and were bled to obtain serum 2, 7 and 12 days thereafter. These sera were given to guinea pigs sensitized with normal horse serum 5 minutes before shock and seemed to protect them from multiple lethal doses of the antigen. All of the untreated controls, the number not being stated, died from smaller doses of antigen, as well as the 1 animal treated with normal rabbit serum. Unfortunately, only 1 animal was reported for each of the treated sera. Similar experiments with guinea pig serum obtained 2 days after ink injection protected one sensitized guinea pig. The serum from a guinea pig bled 12 days after an ink injection and that of an untreated animal failed to protect one guinea pig apiece. From these experiments, the authors concluded that a desensitizing principle was secreted in the blood after an injection of China ink. It does not seem to us that it is necessary to advance such an explanation since the changes could conceivably be explained on some other basis. Nevertheless, the work is of great interest and should be confirmed by larger series.

Simitch, in 1926, was unable to protect animals by 4 intravenous injections of 1 cc. of 3 per cent China ink, 1 day before the shocking dose of antigen. The number of animals in the control and treated series was not given, but all died. It is possible that the amount of antigen used for shock was too high.

Simitch also failed to obtain any protection by giving ink 1 week after splenectomy and 1 day before the *sensitizing* dose of antigen.

Summary. There is no conclusive evidence that anaphylactic shock can be inhibited by the injection of ink given before or during sensitization. The small number of animals used in the experiments makes it impossible to evaluate the results. The work of Moldovan and Zolog, especially their third paper, however, offers an interesting approach which seems worthy of more complete investigation.

Intercurrent infection

This most interesting aspect of the problem has been approached by comparatively few workers, although more study along this line would be of value.

Von Pirquet, in 1908, noted that tuberculous children lost their reactivity to tuberculin for about a week during measles.

Hartoch and Sirenskij, in 1912, found that sensitized guinea pigs, infected with trypanosomes, became insensitive to lethal amounts of antigen. This was explained by the authors on the basis of loss of complement, due to the intercurrent infection. No protocols of antigen titration were given. Of the 5 animals infected with Nagana trypanosomes 12 days after the sensitizing dose of antigen, and given the second injection of antigen 47 or 48 days after the first, 2 died of anaphylactic shock and 3 of infection. The results, therefore, were suggestive, but inconclusive.

Seligmann, in 1912, reported that guinea pigs infected with tuberculosis at the time of their sensitization with papain, resisted 5, 10 or 20 shocking doses of antigen lethal for the uninfected controls when shocked 4 or 5 weeks later. This resistance was not, however, invariable, some of the infected animals dying subacutely from ½ to 2 hours after the shock. The animals with localized tuberculosis died more quickly when fatally shocked, but required from 3 to 5 lethal doses. The more generalized the tuberculosis, the more difficult was it to induce shock; 20 minimum lethal doses of antigen being necessary to kill some of these animals. In animals infected before or after the time of sensitization, there ensued, with the appearance of the tuberculosis, a retrogression of sensitivity, similar to that in the animals infected at the time of sensitization. No protocols were given for this set of experiments. In a confirmatory series, the animals infected with tuberculosis after sensitization with papain were found to resist 3 minimum lethal doses of antigen 31 days later. Some of the infected animals and uninfected controls were bled and their sera used to sensitize normal animals passively. Twenty-four hours later, when all of these animals received 1 cc. of the antigen, the 2 sensitized with the blood of infected animals had no shock, while the 2 controls both had shock, fatal in 1 case. Seligmann, therefore, concluded that the lessening of sensitivity in infected guinea pigs depended on the reduction of specific antibody, not on a lack of complement. He also concluded that tuberculous infection in sensitized animals leads to a far-spread loss of anaphylactic sensitivity.

Friedberger, in 1913, performed experiments similar to those of Seligmann, except that he worked with mild shock in which temperature changes could be noted. The work was carefully controlled and showed that, in general, much more marked temperature changes occurred in the uninfected sensitized controls upon the second injection of antigen, than in the animals which had been infected with tuberculosis at the time of sensitization. The lethal dose of shocking antigen was also found in most cases to be much higher for the infected animals than for the uninfected.

Isaacs, in 1924, made an unsupported statement that acute distemper prevented desensitization, but that this infection in the recovery stage did not.

Wedgewood and Grant, in 1924, observed that young rats on a complete diet could be sensitized if they had an infectious disease of the lungs, although normally such animals are resistant to sensitization. The possible theory to explain this increased sensibility was that the infection exhausted the vitamin B reserves, which ordinarily protected against anaphylaxis.

Lewis and Loomis, in 1926, concluded that the allergic irritability of guinea pigs was *increased* by infection with Brucella abortus or with a streptococcus, or by the injection of dead tubercle bacilli, all of these being less effective than infection with tubercle bacilli. These experiments are not comparable to the others because of differences in technique.

Summary. The studies of Hartoch and Sirenskij, of Seligmann and of Friedberger have indicated a decreased susceptibility to shock in animals given an intercurrent infection. On the other hand, Wedgewood and Grant, as well as Lewis and Loomis, using different methods of approach, have offered evidence of an increased irritability. Certainly more work on this problem is clearly indicated.

Invertin

Doerr, in 1908, cited the use of this without details.

Iodbenzoic Acid

Ambert and Knox, in 1912, obtained no inhibition of the cutaneous reaction in sensitized guinea pigs treated with this drug before the second injection of antigen. This drug was used as a control for experiments with sodium iodoxybenzoate.

Iodine

Besredka, in 1907, was unable to modify the toxicity of serum with Gram's solution.

Anderson and Rosenau, in 1908, found that the addition of 1.5 gram of iodine and 3 grams of potassium iodide to 25 cc. of serum had no effect upon its toxicity. Of 5 controls, given 6 cc. of normal serum subcutaneously, 2 died and 3 recovered after acute shock. Of 12 animals given the treated serum subcutaneously, 4 died, 1 recovered from severe shock and 7 had mild or slight shock. All 3 of the animals died that were given 6 cc. of treated serum intraperitoneally. When iodine was injected subcutaneously 1 or 24 hours before the intracranial injection of the antigen, all of three animals so treated died. Two of the 3 animals so treated 1 hour before the intraperitoneal administration of the antigen died. The third recovered after marked symptoms. Controls were given here only for the subcutaneous injection of the antigen. Earlier controls, however, had shown that 6 cc. of serum, given intraperitoneally, probably represented many lethal doses.

Von Dungern and Hirschfeld, in 1911, concluded that the action of anaphylactic antibodies was strongly raised by iodinization. Three guinea pigs, passively sensitized with untreated anti-sheep serum all died when given sheep serum 24 hours later, while the 3 animals given iodinized anti-sheep serum had mild or no shock upon the subsequent injection of antigen. Experiments with active sensitization showed that after sensitization with normal horse serum, animals shocked with freshly iodinized serum reacted less violently than those shocked with normal serum or old iodinized serum. Unfortunately, the small number of animals used for each variation of test makes the evaluation of these findings difficult. Probably these findings should be interpreted as the result of a chemical change of the antigen.

Summary. There is some, but inclusive evidence, offered by von Dungern and Hirschfeld, that iodinization of the serum antigen may inhibit active or passive anaphylaxis. Their findings are more complete than the earlier, negative work of Besredka and of Anderson and Rosenau.

Iron in state of hydrosol

Henrijean and Kopaczewski in 1925, gave no details of their experiments with iron in this state.

Iron oxide

Simitch, in 1926, failed to inhibit shock by giving a total of 4 cc. of 6 per cent oxide of iron intravenously the day before sensitization. Iron was used alone or 1 week after splenectomy. No titration of the lethal dose of antigen was made. No protocols were given and the number of animals used was not stated.

Iron oxide, saccharated

Petersen, Jaffe, Levinson and Hughes, in 1923, gave dogs daily intravenous injections of 15 cc. of 20 per cent saccharated red oxide of iron during the sensitization period. The conclusion reached was that shock was modified by blocking the reticulo-endothelial system in this way. The authors believed that the so-called reticulo-endothelial blockade did not necessarily mean a diminished permeability of the endothelium, but that there might be either a true blockade of the cells or an increased activity of the endothelial elements, with a more rapid destruction of the antigen and a consequent protection of the parenchymal cells of the splanchnic area.

Michelazzi, in 1927, found that the minimum lethal dose of antigen for 11 controls was 0.025 cc. of serum per 300 grams of body weight. Seventeen animals were given 4 subcutaneous injections of a 2 per cent solution of saccharated iron oxide on the fifth, fourth, third and first days before the sensitizing dose of antigen. The minimum lethal dose of antigen for these animals was 0.05 cc. of serum per 300 grams of body weight, or twice that of the controls. Six animals were given 4 subcutaneous injections of 1 cc. of saccharated iron oxide on 4 consecutive days, starting 24 hours after the first injection of antigen. The minimum lethal dose of antigen for these animals was 0.3 cc. of serum per 300 grams of body weight or 12 times that of the controls. In another series, the minimum lethal dose of antigen for the 7 controls was 0.3 cc. of serum per 100 grams of weight. In the series of 7 animals treated with 4 cc. of drug 4 hours before shock, the minimum lethal dose of antigen was 0.9 cc. per 300 grams of weight, or 3 times that of the controls.

Summary. Michelazzi offered well controlled evidence that the administration of saccharated iron oxide modified shock if given before sensitization or during the sensitization period. Similar results were obtained by Petersen, Jaffe, Levinson and Hughes by treating dogs during the sensitization period.

Kephalin

Karsner and Ecker, in 1924, obtained some evidence of inhibition of shock with kephalin, given in dosages of 0.014 or 0.028 mgm. per gram half an hour before shock. In this series 3 of the 5 animals given the smaller dosage and all of the 5 given the larger dosage survived the shocking dose of antigen lethal for an unstated number of controls. Results were less striking when treatment was given 1 hour before shock. No protection was obtained when treatment was given 24 hours before shock. Although extremely conservative in their conclusions and realizing the possible fallaciousness of their results, on account of the small number of animals, the authors concluded that their results with this colloid were sufficiently encouraging to justify further experimentation.

Lanolin

Fujioka, in 1925, stated that both active and passive anaphylaxis could be prevented by blocking the reticulo-endothelial cells with an emulsion of lanolin. We have been able to read only the abstract of this paper.

Lecithin

Anderson and Frost, in 1910, gave 6 sensitized guinea pigs 0.25 gram of egg lecithin 24 hours before the injection of 5 cc. of horse serum. Five animals had shock, 1 of them dying. The authors did not consider this indicative of any modification of shock.

Banzhaf and Steinhardt, in 1910, obtained no protection by emulsifying lecithin with the shocking dose of antigen. Lecithin given in doses of from 250 to 500 mgm., however, protected sensitized guinea pigs from 5 cc. of horse serum given 20 hours later. No protocols were given for these experiments.

Achard and Flandin, in 1911, found that animals could be protected against shock by giving 0.25 gram of lecithin 2 hours before shock to 11 guinea pigs, or 0.1 gram the day before shock, given to 4 animals. The injection of lecithin 30 minutes before shock attenuated it in 2 cases, but did not modify it in 2 others. An unstated number of controls were shocked. Details were lacking.

Doerr, in 1913, cited previous experiments with both purified and egg lecithin with which he was unable to obtain any protection against anaphylactic shock.

Carpani, in 1920, in a well controlled experiment with a rather small number of animals, injected lecithin subcutaneously, 3 cc. of a 5 per cent solution in olive oil. Protection against shock was obtained when this treatment was given before the second injection of antigen.

Schneider, in 1927, obtained no inhibition of shock by treating animals with both radiation and subcutaneous injections of lecithin. His series was very small.

Summary. There is no striking evidence that lecithin can inhibit shock. The favorable reports of Banzhaf and Sreinhardt, of Achard and Flandin and of Carpani are weakened either by a lack of details or by the use of too small a series of animals. The same criticism applies to the unfavorable work of Anderson and Frost, of Doerr and of Schneider.

Lipoids

For the work done with antitrypsin, kephalin and lecithin, the reader is referred to these headings.

Duprez, in 1922, concluded that lipoids exerted an anti-anaphylactic action. He used the acetone insoluble Wassermann antigen of Bordet. Guinea pigs sensitized with 2 cc. of horse serum were given 2 cc. of the lipoid emulsion intravenously 1 hour before shock. The treated animals had very mild or no shock and an unstated number of controls died. No protocols were given.

Stern and Reiss, in 1922, working with dogs, found, as previously shown, that while the sensitizing injection of antigen caused an increase in the blood lipoids, in anaphylactic shock they were lowered within ten minutes after the injection of antigen. The authors correlated the lipoid content of the blood with its non-coagulability in shock.

Magnesium sulphate

Anderson and Rosenau, in 1908, found that no protection could be obtained by giving 0.1 gram of this drug subcutaneously the day before shocking by the injection of 0.5 cc. of serum in the brain. One pig was used. This was the titrated minimum lethal dose.

Rosenau and Anderson, in 1909, studied the effect of magnesium sulphate given subcutaneously as an anesthetic to sensitized guinea pigs before shock. No inhibition of shock was obtained, whether calcium chloride was used to counteract the effect of the magnesium sulphate or not. Thirty-seven animals were treated with different combinations, the amounts of serum for shock invariably being 6 cc. given intraperitoneally. Probably this represented multiple lethal doses.

Summary. There is no evidence that magnesium sulphate influences anaphylactic shock, but not all of the possibilities have been studied.

Manganese chloride

Pico, in 1924, gave 35 guinea pigs 0.5 cc. of manganese chloride in 0.4 per cent. solution, just before the shocking dose of 1 cc. of antigen. Twelve of these treated animals had mild or no shock and 23 died. No controls were given. Nevertheless, the author stated that the animals had been almost completely protected. It must however, have been difficult to determine the degree of protection in the 23 casualties. Other experiments, done with sub-sensitizing injections of antigen and with manganese chloride treatment during the incubation period, were too small in number, although somewhat controlled, to allow any conclusions to be drawn. The method, however, is of possible interest.

Klopstock, in 1925, in a small, but controlled series, found that none of the 6 guinea pigs treated with 1 mgm. of manganese chloride died when given the second injection of antigen, although 4 of them had severe shock. Four of the 6 untreated controls died and 1 of the survivors had acute shock. The author concluded that manganese chloride protected, but, in our opinion, the series was too small to be more than suggestive.

Summary. Of the 2 articles on the use of manganese chloride, Pico's is uncontrolled and his conclusions do not follow from the data presented. Klopstock's well controlled work presented evidence in a small series of animals that the drug may have an inhibitory action. There is, however, no entirely conclusive evidence in regard to manganese chloride.

Mercurochrome–220

Martin and Hill, in 1930, found that while mercurochrome given intravenously in doses of 5 mgm. per kilogram had no inhibitory action if given early in the sensitization period, when the drug was given the day before the shocking dose of antigen, the treated animals had definitely less shock than the controls. Thus of 51 treated animals, 30, or 58.8 per cent lived and 21 died, while of the 37 controls, 13 or 35.1 per cent lived and 24 died.

Mineral water

Billard, in 1913, sensitized guinea pigs with an unknown amount of antigen and gave one set 6 daily intraperitoneal injections of 2 cc. of Eau de César. A similar set received 2 cc. of Eau de Sain-Mart (Royat). Fourteen days after sensitization, the shocking dose of antigen was given. The sole survivors were the 6 animals treated with Eau de Saint-Mart. The degree of the second injection was not given. No distilled water controls were made.

Chaussevant, Galup and Poirot-Delpech, in 1913, studied transported Vichy water 24 hours old and found no difference between the treated guinea pigs and the controls which had received distilled water. The authors believed, for no stated reason, that if mineral waters desensitized, it was on account of radio active substances contained therein.

Gobert, in 1913, treated guinea pigs with 2 cc. of water from Aïn Sbia (Korbous) for 11 days. He agreed that Chassevant, Galup and Poirot-Delpech that the treated animals, when shocked, behaved like the controls. The series was short but the number of controls, 4, equaled the number treated. However, 3 different shocking doses of antigen were given these 8 animals.

Billard and Grellety, in 1913, studying Vichy water, obtained results similar to those of Billard with Royat water. They used rabbits, sensitized with horse serum, and found that while the animals treated with water from 1 spring Chanel, had almost no shock, those treated from 2 other springs had as much shock as the controls. They therefore concluded that mineral waters could modify anaphylaxis favorably or unfavorably, depending upon the source of the water. No protocols were given.

Billard and Daupeyroux, in 1913, made a brief report of a study of Bourboule water at its source. Twenty-five rabbits were used, 5 controls and 5 in each group given different amounts of treatment. The second dose of antigen was given on 3 different days after sensitization, so that the number of animals used for any given test must have been very small. No protocols were given and it is difficult to evaluate the results, but the authors believed that definite attenuation of shock was obtained in some of the tests.

Mougeout, in 1919, worked with rabbits, continuing Billard's work. He claims to have obtained good results.

Ferreyrolles, in 1919, confirmed the findings of Richet, of Bellin, whom he cites without reference and of Daupeyroux. Ferreyrolles, however, presented none of his own experiments.

Galup, in 1920, summarized the articles to date, without giving further experimental work.

Kopaczewski and A. H. Roffo, in 1920, reported experiments in which the 4 animals treated with mineral water had no shock, while the one and only control died. It is impossible to draw definite conclusions from such a series. On the theory that the sensitizing action of mineral waters was due to the presence of carbonates and bicarbonates, the authors also reported some experiments with these substances, which will be cited under the headings of Sodium Carbonate and Sodium Bicarbonate.

Arloing and Vauthy, in 1921, studied transported Vichy waters hermetically sealed when collected and used 24 hours later. Two controls were used, both of which had extreme shock, 1 dying in 24 hours and the other after 4 days. Of 9 animals treated with mineral waters, 6 had mild or no shock and 3 died. The authors concluded that both Vichy waters tested could attenuate or suppress shock if given in sufficiently large amounts over a long enough time. They also believed that the viscosity of the blood was augmented by these waters.

Arloing and Vauthy, in 1922, in their second paper, gave daily subcutaneous injections of a 4 cc. of various Vichy waters for 21 days. The shocking dose of antigen was "quelques gouttes," given intracranially 24 hours after the last treatment. The treated animals, 6 from each of 4 springs, had slight or no shock, while the four controls, 1 for each set, had severe shock, 3 with recovery. This is certainly a suggestive paper.

Arloing and Vauthy, in 1922, in a third paper, gave Vichy waters with the shocking dose of antigen, which was 0.25 cc. of serum, diluted with either 0.75 or 4.75 cc. of water. The authors believed that they obtained definite protective action from all three sources. The treated series was very small. Of 4 treated animals, from each of 3 springs, all lived, while 1 of the 2 controls died. This was not a striking paper, because only 1 animal was used for each variation of the experiment.

Arloing and Vauthy, in 1923, in a fourth paper, mixed Vichy water with the sensitizing dose of antigen. Five treated animals recovered with slight shock and 5 of the 6 controls died, the other being acutely shocked. In this case, it is possible that the antigen, mixed with the mineral water, assumed another chemical structure and that the second injection of untreated antigenic serum therefore was not specific.

Arloing, Langeron, Milhaud and Ricard in 1923, used sulphur water from Luchon. They claimed that protection was obtained if the guinea pigs had 10 daily subcutaneous treatments with 2 cc. of the transported water or 6 cc. daily for 21 days. They gave no controls. There was no titration for the minimum lethal dose of antigen. Various antigens were used and very few animals were treated. The results cannot be accepted as authoritative.

Cahn, in 1924, repeated the work of the French authors and was able to protect his guinea pigs with Vichy water. The three treated animals that received from 16 to 19 daily injections of Vichy water lived after the shocking dose of antigen lethal for the controls. Using German mineral water from Fachinger, Wildung and Emser, he obtained the same results with Emser water, but no results of any value with Fachinger or Wildung water. Although this experiment was carried out with a small number of animals, the difference between the treated and control animals was marked.

Henrijean and Kopacezewski, in 1925, studied the iron water of Spa and also iron in a state of hydrosol. In the first set, all of the controls died, while all of the six guinea pigs treated with about 10 subcutaneous injections of 0.5 cc. of the mineral water every 2 days for 20 days had very mild shock. Of the animals treated in this way intraperitoneally, 50 per cent died and 50 per cent had mild shock. Other experiments gave similar results with intraperitoneal treatment. When treatment consisted of 1 injection of 2 cc. of the mineral water injected intravenously just before the shocking dose of antigen, all died in 1 set and all had moderate shock in another. It is not clear that additional controls were made with the sets done after the first series as further controls were not recorded. It is not valid to carry over controls from one set to others done at different times, so that the value of the other sets may be questioned. The first set, however, was entirely valid.

No details were given of experiments with iron in the state of hydrosol. The authors concluded that distinct phylactic properties were possessed by the water studied. In spite of their hypothesis that the water acts by augmenting the viscosity of the blood, i.e., in stabilizing the humoral colloids, the authors finally concluded wisely, that the mechanism of desensitization is still to be explained.

Perrin and Abel, in 1927, in an uncontrolled investigation, compared the desensitizing action of a mineral water containing calcium sulphate and equivalent solutions of calcium sulphate.

Salvagnini, in 1927, obtained inconclusive results by treating guinea pigs with water from the hot springs of Abano. With rabbits, however, he obtained some inhibition of shock. Treating guinea pigs with mud from the same region intensified shock. His experiments were carefully controlled and seem valid. His bibliography contained a few references which we have been unable to obtain.

Summary. It is interesting to review the results critically. We find 14 articles reporting upon the helpful effect of continuous injections of certain spring waters in allaying the fatal effects of the shocking dose of antigen.

Actually, 5 of them offer some definite evidence. The first is Billard, who, unfortunately, does not give satisfactory protocols. The work of Arloing and Vauthy in 1922 seems valid. The investigations of Henrijean and Kopaczewski, of Cahn and of Salvagnini were well controlled.

For various reasons, the other six do not fulfill the very definite requirements of validity.

In regard to negative results, the work of Chassevant, Galup and Poirot-Delpech was well done and they were unable to find any protection with transported waters.

Milk

See also Whey Protein.

For the technique of the experiments of *Rusznyák and Korányi*, in 1927, by which some inhibition of shock was obtained by injection of milk, please see "Cascosan."

In 1930, *Martin and Hill* found that the intramuscular injections of milk protein, (Aolan) throughout the sensitization period protected a certain number of guinea pigs from shock.

Morphine

Besredka, in 1907, found that from 14 to 18 centigrams of morphine hydrochloride caused no true narcosis in sensitized guinea pigs and that when shocked, the treated animals died in the same manner as the controls.

Banzhaf and Famulener, in 1908, made a general statement that they could obtain no inhibition of anaphylactic shock with morphine sulphate.

Summary. There is no evidence from the studies of Besredka or Banzhaf and Famulener that morphine has any inhibitory action.

Multiple sensitization

Most of the early work done with multiple antigens was marred by the large amounts of antigen given in both the sensitizing and the shocking doses and also because most of the injections were made intraperitoneally.

However, the work of *Gay and Southard*, in 1908 is decidedly worthy of consideration. They showed that when guinea pigs were sensitized with three antigens, non-lethal reinjection of 1 or 2 of these antigens was apt to desensitize the animal against the third. This phenomenon varied according to the order in which the antigens, egg white, milk and horse serum, were given.

Bessau, in 1911, carried out an experiment which has been more or less repeated by other investigators. The main differences were that Bessau mentions only 1 control and later writers used a few. Bessau, however, used a large dose of shocking antigen (0.5 cc.), given intravenously, which should have been well over the minimum lethal dose. He sensitized his animals with 2 sera and desensitized with small amounts of 1 of them, 0.05 cc. given intravenously, or 1 or 2 cc. given intraperitoneally. Three of the doubly sensitized animals, which received 0.05 cc. of one serum intravenously 5 hours before the shocking dose of the second antigen, all had shock but recovered, only 1 of these animals having a severe reaction. None of the 8 doubly-sensitized animals lived that received the intravenous injection of 1 serum from 1 to 2 days before the shocking dose of the second serum. Of 4 animals which received the desensitizing serum intraperitoneally 1 day before the shocking dose, and 1 each treated 2 and 5 days before shock, all recovered. His small number of animals and the paucity of his controls, render Bessau's report of very little value in itself.

Benjamin and Witzinger, in 1911, found that of 10 guinea pigs given 1 cc. of horse serum 24 hours before sensitization with 0.01 cc. of beef serum, 1 died when shocked with 0.2 cc. of beef serum 9 days later, 3 had severe shock with recovery and 6 mild or no shock. All 7 of the comparable controls for this series died. The authors concluded that anaphylaxis could be inhibited by the injection of serum before sensitization. Within the limit of the number of animals used, this conclusion seems justified. This work should be compared with that of Weil, in 1914, on the inhibition of passive anaphylaxis by the injection of large amounts of normal serum before the administration of immune serum. Weil's study is cited under Normal Serum.

Bauer, in 1912, sensitized guinea pigs with beef serum and hog plasma. He gave 4 of them 3 cc. of beef serum intraperitoneally 2 days before shocking them with different amounts of hog plasma, given intravenously. Two of these animals died, 1 having received 1 cc. of hog plasma, an amount lethal for a normal animal, and 2 had mild or no shock from doses of antigen lethal for the controls. Although carefully controlled, the number of animals used for the experimental variations was too small to allow valid conclusions to be drawn.

Szymanowski, in 1912, repeated in a more detailed manner the work outlined above. He sensitized his animals with 2 antigens and then tried to desensitize with large, sublethal doses of one of them. He was unable to show that the desensitization produced by the first antigen conferred more than a slight resistance to the second. His work was well done, but may be criticized in that not enough animals received the shocking dose of the second antigen to make his estimation of the minimum lethal dose accurate.

Kumagai and Odaira, in 1912, carried out parallel experiments with passive anaphylaxis. When guinea pigs were sensitized with both anti-sheep and anti-human rabbit sera and shocked with 1 antigen and then with the other, the maximum non-lethal dose of the first antigen was found not to protect against the second. Only a few animals were used.

Wells and Osborne, in 1913, found that heterologous proteins failed to give complete protection against the antigen, although animals sensitized with 2 antigens and shocked with one were less sensitive to the second.

Weil and Coca, in 1913, found that after double sensitization, desensitization with 1 antigen rendered the animal only slightly more resistant to the second antigen.

Dale, in 1912–13, came to the conclusion that when animals were sensitized with 2 or more antigens, "desensitization of the muscle with one antigen is not without effect on its sensitiveness to others." This work was done in vitro.

Bessau, Opitz and Preusse, in 1914, sensitized guinea pigs with 2 sera and then determined the lethal shocking doses of both. Of five sensitized animals given sublethal amounts of 1 antigen, beef serum, and then given horse serum, the second antigen, 3 died and 2 lived, but the amounts of the second antigen given were too small to make any demonstration of inhibition of shock conclusive. Experiments were also done with local anaphylaxis in rabbits. It was concluded that in doubly sensitized rabbits the intravenous reinjection of only 1 serum influenced the appearance of local anaphylaxis with either the homologous or the heterologous antigen. It is unfortunate that the number of animals used in some of this rather original work was not greater.

J. H. Lewis, in 1915, demonstrated that the injection of large amounts of 1 type of animal serum or egg white before the serum which was to be used for antigen would definitely protect animals against the intoxicating dose of antigen. This confirmed the earlier work of Benjamin and Witzinger, cited above. Lewis had a rather small number of controls, but his work was carefully done and as accurate as it was interesting.

Massini, in 1918, sensitized guinea pigs with 2 or 3 sera and found that a non-specific antianaphylaxis accompanied the specific desensitzation. His technique made use of excised tissue.

Lumière and Couturier, in 1912, sensitized animals with 2 antigens and after 2 weeks administered subcutaneously sublethal doses of 1 of them. Six and 4 hours later, the second antigen was given intracardially. The controls died and the treated animals lived. The next day the latter received 0.5 cc. of the second antigen and died. The experiment was well done and demonstrated desensitization lasting but a short time.

Brack, in 1921, sensitized guinea pigs with 3 sera. He found that their reaction was strongest to the antigen first used for sensitization and there was a clearly quantitative difference between specific and non-specific antianaphylaxis. He used Massini's technique of studying excised intestinal muscle.

Summary. The work of Wells and Osborne, of Lumière and Couturier, of P. Lewis and of Benjamin & Witzinger is all of value and seems to show that when 2 or more antigens are given simultaneously for sensitization, the subsequent injection of 1 of these antigens will decrease the sensitivity of the animals for the other antigen. Dale, Massini and Brack, by their in vitro work, came to the same conclusion. On the other hand, the well controlled work of Szymonowski, of Kumagai and Odaira and of Weil and Coco, did not produce evidence which could be so interpreted. The work of Bessau and his associates is, unfortunately, not acceptable on account of the lack of controls.

Myrosin

Doerr, in 1908, cited the use of myrosin, but gave no reference or description of the experiments.

Neoarsphenamine

Jungeblutt, in 1928, studied the inhibitory action of this arsenical by treating sensitized guinea pigs before shock. He gave intravenously 1 cc. of a 1:50 dilution of the drug to different animals 5, 15, 30 and 60 minutes respectively before the second injection of antigen. The minimum lethal dose of antigen, determined upon 4 animals, was found to be 0.3 cc. intravenously administered. All of the 4 animals treated 5 minutes before shock died as did 2 of the 4 treated 15 minutes before shock. Three of the 4 animals treated 30 minutes before shock and all the of 4 animals treated 1 hour before also died. Parallel experiments with passive anaphylaxis, in which the above mentioned dosage of drug was given, showed that 3 of the 4 animals treated 15 minutes before shock lived as well as 2 of the 4 animals treated 30 minutes before shock. These experiments in passive anaphylaxis are of doubtful value on account of the small number of control animals. The protective action obtained by Brodin and Huchet by adding sodium formaldehyde sulphoxylate to the second injection of antigen may be cited here.

Nephrectomy

Pistocci, in 1920, could not find that nephrectomy had any influence on anaphylactic shock. Details of his experiments are lacking.

"Novoprotin" and "Novoprotein"

Rysznyák and Korányi, in 1927, obtained evidence of inhibition of shock with "Novoprotin." For the technique of the experiments, please see "Cascosan."

Schneider, in 1927, obtained no inhibition of shock by giving animals both radiation and subcutaneous injections of "Novoprotein." His experiments are not comparable with those of Rysznyák and Korányi, because of the differences in method.

Olive oil

Achard and Flandin, in 1911, gave injections of from 5 to 20 cc. of olive oil before the injection of antigen without any protection.

Opium

Besredka, in 1907-c, found that 1 cc. of 1 per cent opium, although causing narcosis, had no inhibitory action upon shock.

Orange juice

See Vitamin C.

Oxygen

Anderson and Schultz, in 1909, gave artificial respiration with oxygen before the shocking dose of antigen and found that of their 16 treated guinea pigs, 7 animals, or 43 per cent lived, as compared with 3 animals, or 18 per cent of the 16 controls. Combining artificial respiration with treatment with adrenalin and a chloral hydrate urethane solution, some protection was also afforded, but the exact rôle of the oxygen in these tests cannot be determined.

Pancreatin

Anderson and Rosenau, in 1908 gave 1 gram of pancreatin subcutaneously the day before shock. One guinea pig was so treated and died when given 0.5 cc. of the serum antigen in the brain. Some of the controls were killed with 0.25 cc. of antigen.

Lesné and Dreyfus, in 1911, found that, in rabbits sensitized with egg white, shock could be repressed by adding pancreatin to the second injection of antigen. When the antigen was actinocongestin, similar treatment caused inhibition of shock, but less than in the egg white series. No protocols were given and the number of controls was not stated.

Paraldehyde

Rosenau and Anderson, in 1909, gave 3 sensitized guinea pigs 0.9 or 1.0 cc. of paraldehyde by mouth 1 hour before shocking them intraperitoneally with 6 cc. of the serum antigen. One animal died, 1 recovered after marked shock and 1 had mild shock. No controls were given, but the authors concluded that the drug had but slight influence.

Pepsin

Rosenau and Anderson, in 1908 (Hyg. Lab. Bull, No. 45), obtained no inhibition of shock by giving 0.5 gram of pepsin subcutaneously 1 day before the second injection of antigen, which was 0.5 cc. injected into the brain. Only 1 animal was treated. This was apparently the minimum lethal dose of antigen.

Lesné and Dreyfus, in 1911, observed the same effects with pepsin as with pancreatin. Rabbits sensitized to egg white could be protected from shock by adding pepsin to the second dose of antigen. When actinocongestion was used for sensitization, similar treatment with pepsin also inhibited shock, but less strikingly than in the egg white series. No protocols were given.

Peptone

Anderson and Rosenau, in 1908, found that 0.2 gram of peptone given subcutaneously the day before shock caused no inhibition. The antigen was injected into the brain in an amount of 0.5 cc.

Biedl and Kraus, in 1909, in an article without protocols, stated their belief that peptone and the shocking dose of antigen acted in the same way, animals treated with peptone having no reaction to the antigen and vice versa.

Pfeiffer and Mita, in 1910, were primarily interested in temperature variations. They found in sensitized animals that if treatment consisted of the intraperitoneal injection of 0.3 gram of peptone, in a 10 per cent solution, 1 or 2 days before shock, the temperature variations after shock were from 3.2° to 4°C. But if this treatment was given 3 or 12 days before shock, no temperature variation was observed upon the injection of the antigen, the 3 controls showing variations from 5° to 9°C. and all dying. Animals treated with 0.05 gram of peptone 3 or 4 days before shock also had much milder shock than the controls, which died. The authors, therefore, seemed justified in their conclusion that anaphylactic shock could be influenced by peptone.

Pfeiffer, in 1911, found that of 8 animals given 0.25 cc. of 10 per cent peptone and 2 given 0.3 cc. (Table 28, page 633), 7 animals had definitely less shock than the 4 controls.

Kumagai and Odaira, in 1912, found that after intraperitoneal treatment with 0.03 gram of peptone per 100 grams of weight 2 pigs survived 1 or 2 lethal doses of antigen. This treatment did not protect 3 animals against 3 or 4 minimum lethal doses. These authors also did experiments with passive anaphylaxis. Normal guinea pigs were given 0.5 cc. of anti-sheep rabbit serum and 24 hours later 0.1 gram of peptone. Twenty-four hours after the peptone, sheep serum was given intravenously. Of the 4 treated animals, 1 survived 2 minimum lethal doses and another 4, while 1 animal each died from 5 and 10 lethal doses. Unfortunately, there were only 2 controls for this interesting work on passive anaphylaxis.

Heyde, in 1912, obtained less reaction in animals given peptone 3 days before the second injection of antigen than was observed in the controls. No protocols of this work were given.

Besredka, Ströbel and Jupille, in 1913, reported 1 experiment in which 1.5 cc. of 10 per cent peptone was given two hours before shock. Although the peptone itself caused a severe reaction, it failed to protect the animal from the subsequent intravenous administration of the serum antigen. No emphasis can be placed on this work.

Larsen, Haigh, Alexander and Paddock in 1923, obtained no inhibition of shock by giving peptone 30 minutes before the second injection of antigen, or from 5 doses of peptone during the sensitization period. There was no inhibition of response in excised muscle treated with 4 cc. of 4 per cent peptone. Some inhibition of response, however, was obtained with 6 or 8 cc. of this peptone solution and complete inhibition with 10 or more cc. Of the 16 controls for the in vivo experiments, 6 died, 1 had moderate shock, 7 slight shock and 2 no shock. All injections were made intracardially.

Karsner and Ecker, in 1924, treated sensitized guinea pigs with 0.005 or 0.01 mgm. per gram of peptone in 0.1 or 0.2 per cent solutions. When the treated animals were given a shocking dose of antigen lethal for 4 controls, it was found that of 3 animals given the smaller dose of peptone 30 minutes before the antigen, 1 died and 2 had severe shock with recovery. Of three animals given the larger dose of peptone 30 minutes before shock, 1 died and 2 had mild shock. When treatment was given 24 hours before the second injection of antigen, 2 died and 1 recovered from severe shock in both series, i.e., 3 given the smaller and 3 the larger dosage of peptone. The authors interpreted these results as indicating that peptone protected for at least half an hour, but that this action had almost entirely disappeared in 24 hours. In view of the fact that the variation was only 1 pig in each set, that is, treating 30 minutes before 1 of 3 died, and treating 24 hours before 2 of 3 died in each set, such general conclusions do not seem statistically justified without a much larger series.

Summary. Of the favorable reports on the inhibitory action of peptone, those of Pfeiffer and Mita, and of Pfeiffer offer the most convincing evidence. The studies of Karsner and Ecker, of Kumagai and Odaira and of Brodin and Huchet are less conclusive because of the smaller series used, while the articles by Heyde and by Biedl and Kraus are entirely lacking in protocols. Of the unfavorable reports, those of Rosenau and Anderson and of Besredka, Ströbel and Jupille are extremely limited in the number of animals used. The work of Larsen, Haigh, Alexander and Paddock is carefully controlled and shows no inhibition of shock by treating sensitized animals with peptone. They did, however, obtain inhibition of response by adding large enough amounts of peptone to the solution used for the suspension of excised muscle. In short, there is some, but not striking evidence that peptone may inhibit shock within certain defined limits.

Pilocarpine hydrochloride

Levy-Solal and Tzanck, in 1923 gave intracardially 1 mgm. of pilocarpine hydrochloride per 500 grams of body weight simultaneously with the shocking dose of antigen by the same route. All of the 3 guinea pigs so treated lived and the 6 controls died. Three other pigs were given the same dose of pilocarpine a few minutes before the intracardial injection of the shocking dose of antigen. Two lived and 1 died. The author made a general statement that other pilocarpine salts, especially pilocarpine nitrate were without effect.

Stoland and Sherwood, in 1923, in their experiments with excised uterine muscle found that pilocarpine exerted the same stimulation of the sensitized muscle as the antigen.

Pituitrin

Moldovan and Zolog, in 1923, found that pituitrin, given after China ink and 30 minutes before shock, did away with the protective action of the ink.

Potassium chlorate

Belin, in 1911, in a very small series, in which there was usually only 1 control and 1 treated animal for each variation, studied the action of potassium chlorate in guinea pigs sensitized with ass serum. Both controls died, but animals survived that had been treated with from 1 to 4 injections of 2 to 5 centigrams of calcium chlorate subcutaneously. The shocking dose of antigen was given intracerebrally. In other experiments, 0.25 cc. of antigen was given intravenously. Two controls so injected died. One treated animal received subcutaneously 2 doses of 5 centigrams of drug 2 and 4 hours before shock. Another received 10 centigrams of drug by mouth two hours before shock. Both treated animals lived. One animal each treated with 1 or 1.5 cc. of a 1 per cent solution of potassium chlorate, method of administration not stated, given 2 minutes after the shocking dose of antigen lived. Other similar tests were done, but it is impossible to evaluate the results on account of the small number of animals used and the incompleteness of the protocols. There was, however, some evidence that potassium chlorate might inhibit shock. It is possible that a study of larger series would entirely change the findings.

Potassium oxalate

Anderson and Rosenau, in 1908, found that 1 gram of this drug, injected subcutaneously, had no effect when given the day before the shocking dose of antigen, which was 0.5 cc. of horse serum, administered intracranially. Only 1 animal was treated. The amount of antigen used was apparently the minimum lethal dose.

Potassium permanganate

Rosenau and Anderson, in 1906, added 3 cc. of a 1 per cent solution of potassium permanganate to 25 cc. of serum. The mixture was kept for 40 hours at 15°C. and filtered. Three cubic centimeters of the filtrate killed the 1 animal tested.

Belin, in 1911, gave 1 sensitized guinea pig 2 centigrams of potassium permanganate subcutaneously, 1½ hours before the injection of 2 lethal doses of antigen. The animal had acute shock, but recovered. The 1 control died. Another animal, given 0.5 cc. of a 1 per cent solution of drug 2 minutes *after* the shocking dose of antigen also lived, while two controls died.

Pregnancy

J. Duran Reynalls, in 1920, is cited by Lumière and Couturier, 1922, for his statement that sensitized females seemed to lose their sensitivity during gestation.

Lumière and Couturier, in 1921, found that while males die in shock after the second injection of antigen, 0.6 cc., given intracardially, pregnant females had no marked shock. After delivery, sensitivity reappeared. No protocols were given and the number of animals used was not stated.

Lumière and Couturier, in 1922, found that pregnant guinea pigs were resistant to anaphylactic shock and to shock from other causes. The authors introduced ovules of sterile rubber of the volume of the uterine sac into the peritoneal cavity and failed to inhibit shock. From this they concluded that pressure was not the cause of the inhibition in pregnancy. No inhibition was obtained by castration or administration of sexual gland extracts. This protection the authors finally attributed to the increase in blood volume which occurs in pregnancy. No protocols were given.

Pierret and Crampon, in 1923, noted the absence of shock in gravid guinea pigs, but gave no details.

Summary. No definite proof has been offered that animals are desensitized during gestation, although the findings of all of those who have studied this aspect of the problem are in agreement. It is unfortunate that details are lacking.

Pressure

See "Pregnancy." *Lumière* and *Couturier*, 1922.

Quinine

M. I. Smith, in 1920, found that the subcutaneous administration of quinine to sensitized guinea pigs before the administration of the shocking dose of antigen increased their susceptibility to shock.

Rice, polished

See "Vitamin B."

Roentgen ray

Rosenau and Anderson, in 1906, treated the serum used for the shocking dose of antigen with 40 amperes at 4 inches for 40 minutes without modifying its toxicity.

Von Heinrich, in 1913, found that 6 guinea pigs, given an erythema dose of radiation, 3 Kalom, on the day of sensitization, lived when shocked 21 days later, while the 2 controls died. Similarly 1 of 2 animals was protected against 2 minimum lethal doses of antigen, and 1 of 2 against 3 lethal doses, but no protection was obtained against larger amounts of antigen. Treatment with 3 Kalom given 8 days after sensitization seemed to have protected 2 animals. One of 2 animals was protected when this treatment was given 14 days after sensitization. No protection was obtained treating 3 hours before shock. Two guinea pigs, irradiated 14 days before sensitization, survived amounts of antigen lethal for 2 controls. These experiments throughout, however, were insufficiently controlled, no allowance being made for individual variation in the animals. An interesting experiment was done in passive anaphylaxis by irradiating 2 guinea pigs 3 weeks after their sensitization, transferring their sera to normal animals which then had no shock when given antigen, while both of the controls were shocked.

Corper, Black and Moore, in 1922, found roentgen ray treatment without effect. A maximum non-lethal irradiation had no inhibitory action when given 7 days before sensitization, at the time of sensitization, or 7 days before or on the day of the second injection of antigen. Nor was any inhibition obtained by giving repeated small or medium exposures before and during sensitization.

Schneider, in 1927, obtained protection by radiation with a lethal exposure on the day of sensitization or on the 1st and 2nd days thereafter. No protection was obtained with the same treatment during the rest of the sensitization period, or with smaller amounts of radiation. Combining radiation with subcutaneous injections of lecithin or of novoprotein gave no protection in the 3 animals so treated.

Summary. Radiation of the antigen used for shock was found valueless by Rosenau and Anderson. Evidence that exposure of the experimental animals before or at the time of sensitization protects them from subsequent shock has been offered by von Heinrich and by Schneider, although Corper and his associates were unable to demonstrate such an inhibition. Neither von Heinrich nor Corpor, Black and Moore obtained any evidence of inhibition of shock by exposure to Roentgen ray during the incubation period.

Saponin

Kopaczewski and Vahram, in 1919, made a general statement that anaphylactic shock could be inhibited by lowering the surface tension of the blood by an injection of an 0.5 per cent solution of saponin.

Seed extracts

Isaacs, in 1924, worked with seed extracts, or "Proteogens." Of 5 lots of control animals, 20 died and 7 recovered when the shocking dose of antigen was 1 cc. of 10 per cent egg white given intraperitoneally. Of the guinea pigs treated with extract no. 1, 0.015 to 0.25 cc. from 4 to 24 hours before shock, 40 animals died, 2 of these having been starved for 24 hours, and 33 recovered. Treating with extract no. 2, 0.1 to 1 cc. 4 injections, 2 days apart, the last 48 hours before shock, 2 animals died and 1 lived. When 5 injections were given 24 hours apart, the results were the same. Giving single treatments from 30 minutes to 6 hours before shock, 22 animals died and 13 lived. The author concluded that seed extracts produced partial desensitization in over 50 per cent of the animals. The treated animals that died behaved like the controls. The treated animals that survived also showed some shock symptoms, but less than the controls. Isaacs also believed that there was an optimum for each extract and that desensitization, developing during the first hour after treatment, lasted for 24 hours or longer.

Serum fractions

Besredka, in 1908, found that 0.25 cc. of "petit serum," given intraperitoneally a few hours before the second injection of the whole serum antigen, protected guinea pigs. Such protection was obtained with serum treated with 2 parts of alcohol to 1 of serum at 90°C., precipitated with physiological water and filtered or dialyzed. Treatment by rectum and large doses by mouth were also effective.

Dale and Hartley, in 1916, found that "An effective dose of any of the proteins to which the guinea pig plain muscle has been sensitized partially or completely desensitized it to the other proteins of the same serum."

Suarez, in his first paper, in 1928, demonstrated the separate antigenic action of pseudoglobulin, of euglobulin and of serum albumin.

Suarez and Schaefer, in 1928, sensitizing guinea pigs with whole serum and treating them with serum fractions, found that in the series given from 1 to 3 injections of euglobulin from 1 to 4 days before shock, none of the 3 treated animals lived. One animal given 0.25 cc. of pseudoglobulin intra-arterially 2 days before shock survived 2 lethal doses, while another animal, given 2 injections of serum deprived of euglobulin, 5 minutes later survived 4 lethal doses of whole serum. Animals given 1 injection of serum albumin, 1 with toxic and 4 with atoxic preparations, all survived from 2 to 8 lethal doses, treatment being given in amounts of from 0.5 to 3 cc., by various routes. No protocols for the controls were given, but evidently the lethal dose had been determined.

Three animals, sensitized with euglobulin and desensitized with pseudoglobulin or serum albumin, lived after receiving 2 or 5 lethal doses of euglobulin. Two animals specifically desensitized with euglobulin died after receiving 1 to 2 lethal doses. Three animals, sensitized with pseudoglobulin, and treated with from 1 to 2 cc. of serum albumin or euglobulin survived 1 or 2 lethal doses. Of the 2 animals specifically desensitized with 0.25 cc. of pseudoglobulin, 1 survived 2 lethal doses and 1 died after 4. Animals sensitized with serum albumin and treated with pseudoglobulin or euglobulin, survived 4 and 2 lethal doses respectively, while the one given serum albumin as a control survived 3 lethal doses.

Summary. There is some evidence that the administration of serum fractions will inhibit shock. The evidence is not entirely conclusive on account of the small number of animals used and of the lack of protocols.

Serum globulin

Bauer, in 1912, sensitized guinea pigs with 1 cc. of whole milk intraperitoneally and 36 days later treated 5 of them with from 0.01 to 5 cc. of beef serum globulin intraperitoneally. The next day they received 4 cc. of casein intravenously. Two controls, given the same amount of antigen died. The 5 treated animals lived, but were shocked.

Serum, heterologous

Rosenau and Anderson, in 1906, obtained little or no protection by giving horse serum to guinea pigs previously sensitized with dog, hog, beef, sheep, cat or rat serum. With 1 exception these animals all had shock upon the subsequent injection of the specific antigen.

Pfeiffer and Mita, in 1910, studied the effect of the injection of an heterologous serum upon the temperature drop observed in sensitized guinea pigs after the administration of small shocking doses of antigen. In 1 set of 5 animals, with 1 control, 2 cc. of heterologous serum, given intraperitoneally just prior to the specific antigen, might have accounted for the stability of the temperature curve. The temperature of the 1 control dropped 3.6°C.

Ascoli, in 1910, sensitized guinea pigs to anti-diphtheria horse serum. From 14 to 75 days later, he injected retro-ocularly or intracerebrally 0.2 cc. of anti-diphtheria sheep serum and about 50 per cent of the animals lived. With these as controls, he then essayed the same type of experiment with another set and obtained better results, as all of his animals lived. From 15 minutes to 6 hours after this second injection, he gave intracerebral injections of normal horse serum, to determine whether or not his animals had been desensitized by the sheep serum and found that 3 of the 5 animals lived. This was hardly a conclusive experiment. In another series, he sensitized animals with normal sheep serum, desensitized them with 0.2 to 0.5 cc. of horse serum, given either intra-ocularly or intracerebrally and then found that a second injection of sheep serum killed only 29 per cent. Unfortunately Ascoli had no controls for this experiment, which otherwise would have been of value.

Calvary, in 1911, sensitized dogs to horse serum and gave them beef serum before the second injection of antigen, so protecting the animals from shock. The controls were not given.

Benjamin and Witzinger, in 1911, carried out a series of experiments in which horse serum was given 24 hours before shock to animals sensitized with beef serum. It was found that while 5 or 62.5 per cent of the 8 controls died and 2 had severe shock with recovery, of the 17 treated animals, 7 or 41.1 per cent died and 4 had severe shock with recovery. The series in which treatment was given 3 or 4 days before shock had only 1 control.

Weil, in 1913, brought out an excellent piece of work. His number of control animals was small, but they died, having received substantially smaller doses of antigen than the treated animals that lived. His work may be divided into two parts, dealing with, first, passive sensitization and, second, active sensitization.

The daily injection of several large doses of normal sheep serum and normal rabbit serum into guinea pigs prior to the administration of immune rabbit serum (horse) protected the animals against passive sensitization. The author did not show how much protection was gained. This protection was manifest within 1 to 2 days after preliminary treatment had ended and continued for at least two weeks.

"When pigs are actively sensitized with normal horse serum they are not desensitized by 3 successive injections of 3 cc. of sheep serum given subcutaneously at the end of the period of incubation." In this experiment he used 6 animals. They were shocked with 0.2 to 0.3 cc. of antigen and all died, the controls dying after 0.1 to 0.2 cc. of antigen, always given intravenously.

Brack, in 1921 stated that the injections of an heterologous serum in highly sensitized animals could produce anaphylactic shock. This non-specific shock was less than the specific, but could cause a lowering of sensitivity to the homologous serum. He used Massini's technique of studying excised muscle.

Karsner and Ecker, in 1922, tried the effect of giving various dosages of heterologous sera at different times before the shocking dose of antigen. Sensitization was with either human or horse serum. Horse, beef, human and hog sera were given intraperitoneally and subcutaneously for treatment. The animals were divided into 5 sets of 10, 16, 9, 8 and 9 pigs. Four of these sets had two controls and the other set had one. The controls cannot be grouped as the experiments were done on different days and at various intervals after sensitization. It seems superfluous to insist upon more than this number of controls per set. Out of 43 treated animals, 29 lived and 14 died, but as in each set 3 or 4 various methods of desensitization were attempted and as time intervals varied not only between the sets but among the animals in the same set, there are too few animals to permit conclusions to be drawn, although this work is certainly interesting and suggestive.

Summary. No critical piece of work has been done which conclusively demonstrates that heterologous sera, injected before the shocking dose of antigen, will completely or partially protect against specific shock animals which have been actively sensitized with one protein. The work of Karsner and Ecker cannot be accepted as they have no adequate controls. In passive desensitization the work of Weil is of importance and should be repeated. He has shown that passively sensitized animals may be desensitized by the injection of heterologous sera.

There is also no definite work except that of Rosenau and Anderson, showing that protection is not to be gained by the injection of non-specific sera before the second dose of antigen. The work of Ascoli and Calvary does not offer conclusive evidence on either side, nor does that of Brack indicate that heterologous serum may actually cause shock.

Normal serum

See also Heterophile Antigen.

Friedberger and Hartoch, in 1909, obtained no protection by giving sensitized guinea pigs 2 cc. of normal guinea pig serum just before shock with what was probably several lethal doses of antigen.

Dale and Kellaway, in 1923, partially protected 1 guinea pig from shock by giving normal serum 70 minutes before the second injection of antigen.

Weil, in 1914, made further studies of his interesting observations that from 1 to 6 cc. of normal rabbit serum would protect an animal against passive sensitization with anti-rabbit immune serum. This reaction was not specific and human serum, given in this way, protected against anti-rabbit and other sera. This phenomenon Weil called "anti-sensitization" in distinction from desensitization or anti-anaphylaxis. He also found that guinea pigs, sensitized with sheep serum, had no reaction to or desensitization by normal rabbit serum. Animals sensitized with human ascitic fluid had no marked reaction to normal rabbit serum, but could be desensitized by it for 3 days.

Kellaway and Cowell, in 1922, sensitized guinea pigs with horse serum albumin and endeavored to desensitize them with pooled normal guinea pig serum. Three controls given the minimum lethal dose died. Of the 6 treated animals, 2 received 3 cc. of normal serum before the shocking dose of antigen. Of these 1 survived 2 minimum lethal doses and 1 died after 4 minimum lethal doses. Animals were then given 3 cc. of serum 2, 3½ and 22 hours before the minimum lethal dose, or multiples thereof. The animals given 4 lethal doses 2 hours after treatment died, whereas the pig given 2 lethal doses 3½ hours after treatment had no shock. Twenty-two hours after treatment, 1 pig had slight shock from 1 lethal dose and 1 pig died from 2 lethal doses. The series was too small for very definite conclusions but the paper is of interest and should be confirmed. The authors concluded that the maximum protection was obtained by treatment 1 hour before the shocking dose of antigen.

Summary. The work of Weil, less conclusively corroborated by that of Kellaway and Cowell, offers strong evidence that normal serum may inhibit shock under certain conditions. Normal serum controls should be made in all studies where any type of serum is used for treatment.

Sodium bicarbonate

Kopaczewski and Roffo, in 1920, had no shock in sensitized guinea pigs treated with 5 cc. of 10 per cent sodium bicarbonate intravenously or with 10 cc. of a 10 per cent solution given subcutaneously, either 30 or 60 minutes before the shocking dose of antigen. Only 1 control was made so that the findings were not conclusive.

Eggstein, in 1921, contributed an excellent paper, quite the best of them all in our opinion, as the experiments were sufficiently controlled and the series sufficiently large to allow conclusions to be drawn. Treatment consisted of 0.1, 0.3 or 0.4 gram of drug given intravenously to sensitized guinea pigs from 10 to 20 minutes before the shocking dose of antigen. While all of the controls given comparable amounts of antigen died, the mortality among the treated animals was from 78.5 to 92.8 per cent, that is, there was a definite balance in favor of the treated animals. It is possible that the amount of the shocking dose of antigen was unnecessarily large.

Arloing and Vauthey, in 1921, found that 0.5 per cent sodium bicarbonate injected for 10 or 20 days during sensitization, could inhibit shock. The series, however, was small and there were not enough controls. The shocking dose of antigen, 0.25 cc. of serum, was injected into the sub-arachnoid space.

Combiesco and Brauner, in 1928, studied the desensitizing action of saturated solutions of bicarbonate of soda in passive local anaphylaxis of the rabbit. They obtained weaker skin reactions in sensitized animals after 10 cc. of saturated solutions of sodium bicarbonate had been given intravenously, or when the drug was given by mouth, than in the controls. The antigen was administered intradermally.

Summary. All four articles, 3 dealing with general shock and 1 with skin sensitization have given evidence of the inhibitory effect of sodium bicarbonate on shock. Two of the studies, the paper of Combiesco and Brauner on skin reactions and the general paper of Eggstein were sufficiently controlled to warrant the conclusions.

Sodium carbonate

Kopaczewski and A. H. Roffo, in 1920, found that 3 cc. of a 50 per cent solution of sodium carbonate given intravenously, or 5 cc. of this concentration injected subcutaneously 30 minutes before shock, inhibited anaphylaxis. No protection was obtained by the intravenous injection of 5 cc. of a 10 per cent solution or when 5 cc. of the 50 per cent solution was given subcutaneously 2 hours before shock. Only 1 control was given for entire experiment.

Sicard and Paroff, in 1921, found that 12 guinea pigs, given 1 cc. of a 10 per cent solution of sodium carbonate intracardially just before the intracardial injection of the second dose of antigen, had no shock. Of the 12 untreated controls given the shocking dose of antigen in the same way, 9 died at once and 3 had shock, but recovered. Results were less conclusive in the series of 3 animals given sodium carbonate after the second injection of antigen, at the onset of shock, as two of these animals recovered and 1 died.

Sicard and Paroff, in 1921, in experiments without protocols or controls concluded that the intravenous injection of 2.5 per cent sodium carbonate or the addition of 0.3 to 0.4 gram of the drug to serum given intravenously would prevent serum sickness.

Sodium chloride

Heilner, in 1908, conducted a few experiments in rabbits which had a sensitization of period of from 1 to 3 months. Treatment was with 4 per cent salt solution, given to 4 rabbits, 3 of which apparently died from the toxic effects of the salt. The technique employed made the experiments not comparable with those of others.

Friedberger and Hartoch, in 1909, studied the effect of small and large doses of sodium chloride upon shock. Of the two series of experiments with small salt dosages, the first was inadequately controlled with 1 animal and the results inconclusive. The second series, controlled with 4 guinea pigs, all of which died, was marred by the small number of animals, 1 or 2, used for each variation of the treatment, so that while 2 of the 5 treated animals lived, 1 given 0.3 cc. of saturated salt solution intravenously just before shock and the other intravenous injections both before and after shock, the results were not striking.

The series with larger amounts of salt were better controlled, but 1 cc. or more of a saturated solution of salt given intravenously was itself lethal, so that the 5 animals treated with this dosage or more, although seemingly protected from shock, eventually died from the salt. Using smaller amounts, 0.5, 0.6 and 0.7 cc. of saturated solutions of salt, similar toxic effects were obtained, but there was definite protection of 5 guinea pigs from shock, as compared with the death of 7 controls.

A few experiments were carried out with passive anaphylaxis, guinea pigs being sensitized with anti-sheep rabbit serum. The 4 controls died when given sheep serum and 2 of the 5 animals lived that were treated with saturated salt solution before they received the antigen.

Friedberger and Hartoch concluded that sodium chloride inhibited anaphylactic shock, and some of their evidence was conclusive within the limits of error for the number of animals used.

Freidberger and Goldschmidt, in 1910, showed that antigen-antibody union in vitro was not diminished by hypertonic salt solution. They concluded that the inhibition of anaphylactic shock by strong salt solution did not depend upon interference with this union.

Loewit, in 1911, confirmed the findings of Friedberger and Hartoch in a very small, but carefully controlled series of guinea pigs given salt before shock.

Friedberger and Langer, in 1912, could repress shock with large amounts of sodium chloride given by mouth, to both fed and starved guinea pigs. Some of the animals died from the effects of the salt. While all 6 of the controls died, of the 12 animals given from 1 to 4 cc. of concentrated salt solution before the second dose of antigen, 6 died from shock, 4 from the effect of the salt, 1 probably from this cause and 1 animal lived. The animals dying from salt toxicity had no pronounced shock symptoms. Two animals died that were starved before shock, while animals both starved and given salt before the same amount of antigen had no anaphylactic shock, but died from the effects of the salt.

Armand-Delille and Launoy, in 1912, did not agree with Friedberger and Hartoch. The French workers obtained no modification of shock if lethal doses of antigen were given. When sub-lethal doses of antigen were used, shock could be prevented by giving 0.01 gram of sodium chloride per 100 grams of body weight. Protocols of 4 animals were given.

Bornstein, in 1912, presented no protocols on desensitization, but explained the inhibition of shock with sodium chloride as due to an increase in the blood volume.

Ritz, in 1912, in a good paper, concluded that sodium chloride could inhibit shock. Of 9 controls, 6 died at once after the intravenous injection of the shocking dose of serum. Of 5 animals which were injected intravenously with 1 cc. of 30 per cent salt solution just before shock, 2 had shock with recovery, and 2 very mild shock. The fifth treated animal died 7 minutes after receiving a larger amount of antigen than the other animals. 0.7 cc. of 30 per cent salt solution failed to protect 1 animal. Two animals, given 1 cc. of 10 per cent salt solution, had shock but recovered. Of 2 animals given 1 cc. of 5 per cent salt, 1 died and the other had acute, sub-lethal shock. Two guinea pigs, given 1.5 cc. of 1 per cent salt solution, both died when shocked. The best results evidently were obtained with more concentrated solutions of salt given in larger amounts.

Friedberger, in 1913, in experiments on the action of salt in anaphylatoxin experiments may be cited here for his conclusion that the action of salt on complement is the essential factor.

Galambos, in 1913, used in general only 1 animal for each variation of his experiments. The injection of 5 cc. of physiological salt solution 18 minutes before the shocking dose of antigen did not protect 1 animal. Three cubic centimeters of salt solution was given at the same time as the antigen to 1 animal which had shock but recovered. This amount of salt, or 3.5 cc., did not protect 2 animals against a slightly larger amount of antigen. His results with sub-lethal amounts of antigen, given after from 3 to 5 cc. of salt, cannot be evaluated. The experiments were entirely inconclusive.

Dale, in 1913–14, explained the protection of strong salt solution as due to the depressant effect of raised tonicity of the Ringer's solution on the response of plain muscle to stimulant drugs.

Zinsser, Lieb, and Dwyer, in 1915, in work on the preventive action of sodium chloride on proteotoxin shock, worked primarily with anaphylatoxin shock. But their emphasis of the fact that the protective action of sodium chloride is limited should be noted. They were of the opinion that if the shocking dose of antigen or of proteotoxin was much more than the minimum lethal dose, the poisoning was so acute that the protective effects of the salt were entirely masked. This observation is applicable to all of these experimental studies on the non-specific inhibition of anaphylaxis.

Richet, Brodin and Saint-Girons, in 1919, studied the effect of diluting the shocking dose of horse serum with different concentrations of sodium chloride. Of 5 dogs given undiluted plasma, 4 died and 1 had severe sub-lethal shock. Of 2 dogs given plasma diluted with 9 parts of 0.4 per cent salt, 1 died and 1 had acute sub-lethal shock. Two dogs survived severe shock when the antigen was given with a half-volume of a solution of salt "à 200 pour 100." When the antigen was diluted with isotonic salt, animals regularly survived, and had only slight symptoms. Of three animals given antigen diluted with 2 per cent salt, 1 died and 2 lived without shock. The authors concluded that sodium chloride impregnated the nerve cells and immunized them against the anaphylactic poison. No proof to support this hypothesis was presented. Their conclusion, however, that 0.8 per cent salt solution diluting the antigen to 9 times its volume reduced shock seems justified, as similar tests with water and glucose were negative. An elaboration of this report was published in 1920.

Arloing and Vauthy, in 1922, in their third paper, found that diluting 0.25 cc. of the serum antigen with 0.75 cc. or with 4.75 cc. of 0.7 per cent salt solution had no inhibitory effect in the 2 guinea pigs so treated. These were primarily controls for similar experiments with mineral water.

Karsner and Ecker, in 1924, in their study of the colloidal inhibition of shock made the general statement that "large doses of salt solution were effective, but in the small number tried, not so much so as is kephalin."

Wedgewood and Grant, in 1924, showed that 4 young rats, kept on salt-free diet during sensitization, did not respond more to the second injection of antigen than did the 8 controls.

Summary. Of the reports which indicate some inhibitory action of sodium chloride, the most conclusive are the series of animals given large doses of salt reported by Friedberger and Hartoch, confirmed by Loewit, the report of Friedberger and Langer on giving salt by mouth, Ritz' paper and the study of Richet, Brodin and Saint Girons on the dilution of the shocking dose of antigen with salt. The unfavorable reports are those of Armand-Delille and Launcy, who used a very small series of animals, as did Arloing and Vauthy. The papers by Heilner, by Galambos and the series of animals given small doses of salt reported by Friedberger and Hartoch cannot be evaluated. It seems evident, therefore, that under certain conditions, anaphylactic shock may be inhibited by sodium chloride.

Sodium citrate

Rosenau and Anderson, in 1906, obtained no inhibition of shock by adding this drug to the second dose of serum. One per cent of the drug was added to the whole blood at the time of bleeding. Six cubic centimeters of such citrated serum killed both animals tested, but this may have been many lethal doses.

Van de Carr, Lyons and Williams, in 1928, found that 2 per cent sodium citrate, given intravenously to sensitized guinea pigs, altered the colloidal state of the blood sufficiently to greatly increase the degree of anaphylactic shock.

Sodium cyanide

Amberg and Knox, in 1912, sensitized rabbits by combined intravenous and intracutaneous injections of horse serum and studied sodium cyanide for its oxidative effect. They found that it caused an initial increase in the reaction which later became less distinct.

Sodium formaldehyde-sulphoxylate

Brodin and Huchet, in 1921, sensitized guinea pigs with horse serum and found that 1 month later a second injection of 1 cc. of undiluted serum given intracardially, killed 4 of the animals. This seems a very high dose of antigen to employ by this route. The remaining 18 animals received increasing amounts of drug added to the shocking dose of antigen. At a concentration of 25 per cent of drug in 1 cc. of serum, only 1 animal in 11 died. At lower concentrations, more animals died. Similar results were obtained in dogs. This work is worthy of consideration. The authors were able to give as high as 1 gram per kilo to rabbits without ill effects. The relation of this drug, $CH_2OH—SO_2Na$ to novarsenobenzol should be considered in view of the inhibitory effect of the latter.

Sodium glycocholate

Kopaczewski and Vahram, in 1919, found that shock could be suppressed by the injection of 1 cc. of a 1 per cent solution of sodium glycocholate. The number of controls was not stated.

Sodium hydroxide

Ambert and Knox, in 1912, used this to control experiments with sodium cyanide and found more reaction with the cyanide than with the hydroxide.

Doerr, in 1912, cited without reference other work with sodium hydroxide which we have been unable to trace.

Sodium hyposulphite

Lumière and Chevrotier, in 1920, found that their control group of sensitized animals, the number of which was not stated, all died after the second injection of the serum antigen, 0.5 cc. which, with 0.5 cc. of physiological salt solution, was injected intracardially. When 0.5 cc. of 5 per cent sodium hyposulphite was used to dilute the antigen instead of salt solution, none of the animals had shock symptoms. The number treated was not given. The same results were obtained in a confirmatory series.

Sodium iodoxybenzoate

Amberg and Knox, in 1912, working with skin tests in sensitized rabbits found that 10 cc. of N/20 iodoxybenzoic acid, given intravenously, caused a dimunution of the skin reaction. Controls with iodbenzoic acid and benzoic acid, showed no reduction.

Sodium nitrite

Anderson and Rosenau, in 1908, found that the subcutaneous administration of this drug had no inhibitory effect. One guinea pig had 0.3 gram of the drug and 1 had 0.025 gram 3 minutes before 1 cc. of the serum antigen was given intraperitoneally. Both animals died. The drug was used on account of its methaemaglobin production.

Sodium oleate

Jobbling and Petersen, in 1914, found that sub-lethal doses of soap solutions, injected simultaneously with the second injection of antigen prevented shock. This inhibition was obtained with serum albumin, but not when the antigen was whole serum.

Kopaczewski and Vahram, in 1919, treated 8 sensitized guinea pigs with intravenous injections of sodium oleate, given 5 minutes before shock. Two of the animals died, and the rest had mild or no shock. An unstated number of controls died after 0.3 cc. of antigen. The treated animals received 0.5 cc.

Sodium persulphate

Belin, in 1911, found that 1 injection of 5 centigrams of this drug subcutaneously caused sensitized guinea pigs to die faster than the controls when shocked. The number of animals treated was not stated and the number of controls was inadequate.

Sodium sulphate

Anderson and Rosenau, in 1908, obtained no inhibition of shock by giving 0.1 gram of this drug subcutaneously the day before the shocking dose of antigen, which was 0.5 cc. of serum, injected into the brain. Only 1 animal was treated.

Sodium taurocholate

Kopaczewski and Vahram, in 1919, believed that they could suppress shock by injecting 1 cc. of a 1 per cent solution of sodium taurocholate. The theory of its action was that of lowering the surface tension of the blood.

Splenectomy

Pistocchi, in 1920, could not find that splenectomy had any influence on anaphylactic shock. He gave no details, as to antigen, time of operation or the number of animals used.

Simitch, in 1926, considering splenectomy a satisfactory method of blocking the reticulo-endothelial system, failed to obtain any protection in animals splenectomized 1 week before sensitization and treated with either China ink or iron oxide 1 day before sensitization. No determination of the minimum lethal dose of antigen was made.

Klinge, in 1927, studying the Arthus phenomenon, found that splenectomy did not hinder it. No protocols were given in this brief summary.

Starvation

Argaud and Billard, in 1911, presented the hypothesis that inanition for several days before shock should attentuate it. Their reason for this view was that they had observed a disappearance of the white blood cells in starvation, while Lassablière and Richet had noted a marked leucocytosis in sensitized animals. This theory was given without confirmatory experimentation.

Konstantoff, in 1912, kept sensitized animals on water only for 4 days before shock. The experiments were adequately controlled and showed differences in favor of the treated animals. However, the number treated in any given group was small. Three antigens were used and 3 doses of each antigen.

Friedberger and Langer, in 1912, failed to protect 2 sensitized guinea pigs starved before shock, but did get protection if starvation and salt treatment were combined, and inanition being entirely unnecessary.

Pierret and Crampon, in 1923, found that while their untreated controls died at once after the intravenous injection of 1 cc. of undiluted serum antigen, the animals fasted for 36 hours before the same amount of antigen had slight shock, none dying. The same results were obtained with guinea pigs given for several weeks a "regime carancé," i.e., almost entirely dry hay. Only a brief note was presented, without protocols or information as to the number of animals studied.

Zolog, in 1924, disagreed with Pierret and Crampon, finding no difference in the degree of shock in guinea pigs on a normal diet and those kept on water only for 24 and 48 hours before shock. The minimum lethal dose of antigen was calculated per 100 grams of weight of animal, a fallacious method.

Summary. There is no entirely conclusive work to show that starvation can modify shock. The work of Konstantoff is by far the best, but is marred by the many variations, which reduce by too much the number of animals given any particular treatment.

Stovain

Kopaczewski, Roffo and Roffo, in 1920, found that 0.25 cc. of 2 per cent stovain given intravenously just before 0.5 cc. of the shocking dose of antigen protected "all" of the treated animals, while "all" of the controls died.

Strychnine

Doerr, in 1908, cited the use of strychnine without details.

Succinic acid peroxide (alphozone)

Anderson and Rosenau, in 1906, found that 1 or 2 cc. of a 10 per cent solution of this drug added to 25 cc. of serum had no effect upon its toxicity.

Sulphur

Arloing, Langeron, Milhaud and Ricard, in 1923, gave no details of their experiments with colloidal sulphur.

Ruszynák and Korányi, in 1927, observing temperature changes in sensitized guinea pigs, believed that the injection of sulphur, probably a protein compound inhibited shock. The technique of these experiments is given under "Cascosan."

Surgical interference, general

See also Adrenalectomy, Brain Surgery, Nephrectomy, Splenectomy, Thymectomy, Thyroidectomy and Vagus Section.

Dale, in 1912–13, found that excluding the abdominal viscera and the brain from the circulation did not inhibit anaphylactic response of the bronchioles of sensitized guinea pigs.

Manwaring, in 1910, ligated the aorta and vena cava above the diaphragm and found that the upper half of the sensitized animals generally did not react to the shocking dose of antigen. He also removed the gastro-intestinal tract from the pylorus to the rectum, including the pancreas, without obtaining any protection. Removal of the stomach, spleen, kidney, adrenals, ovaries and uterus also failed to protect. Liver extirpation resulted, upon the second dose of antigen, in very slight shock in 7 dogs, no shock in 4 and definite but slight shock in 2. It is obvious that the primary object of these experiments was not to desensitize but to locate the site of the anaphylactic reaction.

Voegtlin and Bernheim, in 1911, found that if the liver was excluded from the circulation, no shock occurred in sensitized dogs.

Talliamine (terpine ozoné)

Belin, in 1911, treated a few guinea pigs with this drug and concluded that subcutaneous or intravenous injection of it inhibited shock if given before the second dose of antigen. Treatment immediately after shock was thought to have prolonged life. The series was too small and the controls too few to make these experiments conclusive.

Temperature reduction

Friedberger and Kumagai, in 1913, immersed sensitized animals before shock for 4 minutes in water at 10°C. While 0.025 cc. of antigen killed 4 untreated controls, 1 of the treated animals survived this dosage, two survived twice this amount, 1 of 2 survived 0.07 cc. of antigen, 1 survived 4 minimum lethal doses and one 5. The authors, therefore, concluded that there was a definite lessening of sensitivity by this treatment. The series was short, but valid as far as it went. In another series, the lethal dose for 4 controls was 0.0075 cc. of the antigen. Treatment was 3 minutes in water at 10°C. before shock. Of the 5 treated animals, 1 survived 2 lethal doses, and one 0.037 cc. of antigen. Seven and 10 lethal doses, however, killed 3 treated animals. Immersion in ice water at 2°C., for 1 to 1½ minutes after shock was given to 3 animals; of these, one survived 2 lethal doses, 1 living and 1 dying after 1 lethal dose. There is some evidence that the authors were justified in concluding that cooling before shock protected the treated animals, but their conclusion that cooling after treatment also protects is much less valid. It should be born in mind that larger series of controls and of treated animals might have yielded entirely different results.

Experiments with passive anaphylaxis showed that 4 of 7 controls died that were given 0.05 cc. of sheep serum 24 hours after the intraperitoneal injection of 0.5 cc. of anti-sheep serum. Five of 6 other animals died that had been similarly prepared and given ice water after the sheep serum so that these results were far from striking.

Thorium-X

Corper, in 1919, obtained no desensitization with thorium-X. The drug was given after sensitization with egg white, horse serum or milk. All of the treated animals in the egg white set died. In the serum set, the results with thorium-X may have been worse than the controls. In the milk series, the controls lived.

Chessevant, Galup and Poirot-Delpech, in 1913, attributed the desensitizing action of mineral water to the presence of radio-active substances. The authors gave no evidence for this theory.

Thymectomy

Parhon and Ballif, in 1923, sensitized 4 thymectomized animals with 0.02 centigrams of sheep serum. Twenty days after sensitization, these 4 animals and 5 controls received 0.1 cc. of shocking antigen. All of the animals were severely shocked, all of the treated animals died and 1 of the controls lived. This work was repeated with similar results. In another paper, 1923, these authors cited experiments in which no protection was obtained in 10 thymectomized guinea pigs, which had been sensitized 20 days after operation.

Thyroidectomy

Pistocchi, in 1920, found that thyroidectomy would give protection against anaphylaxis in about 50 per cent of his animals. He gave no details as to dosage or type of antigen, or as to the time of operation.

Lanzenberg and Képinow, in 1922, from clinical observations concluded that the thyroid gland had some relation to certain aspects of immunity. Following thyroidectomy guinea pigs were sensitized with 1 cc. of 1 per cent serum subcutaneously. The controls (number not stated) were killed with 1 cc. of 10 per cent serum given by way of the carotid artery. Of the 8 thyroidectomized animals, 6 had very little if any shock and lived. One of the 2 that died was found to have remaining fragments of thyroid tissue.

Removal of one half or more of the thyroid gland had no effect upon sensitization. No more did thyroidectomy after sensitization. The work of these authors was well controlled and seems valid.

Képinow and Lanzenberg, later in 1922, showed that if thyroidectomized guinea pigs received an homologous or heterologous immune serum, they would go into fatal anaphylaxis upon receiving the specific antigen. However, a thyroidectomized guinea pig or rabbit, after an injection of horse serum, was unable to elaborate a substance which would confer passive sensitization on other animals. This work was also well controlled.

Képinow, in 1922-b fed thyroidectomized guinea pigs on thyroid extract during the period of sensitization. Six of them died in shock after the second dose of antigen, consisting of 1 cc. of a 1:10 dilution of serum. Three thyroidectomized animals, that had received no thyroid extract had not the slightest shock.

He also fed to 3 thyroidectomized rabbits thyroid gland, during the 21 days they received 15, 10 and 10 cc. injections of normal horse serum at 7-day intervals. The unpooled sera of these animals was injected into 14 normal guinea pigs. Following the usual shocking dose of antigen, all were markedly anaphylactic and but 2 of them lived.

In a later article, (1922), Képinow said "ainsi, le sérum des Lapins thyroidectomisés, injectés avec du sérum de Cheval, bienque ne possédant pas de propriétés anaphylactisantes, renferme presque la même quantité de précipitines que le serum des Lapins normaux. Il en est de même pour les hémolysines."

Appelmans, in 1922, tried to repeat the work of Kepinow and Lanzenberg. He concluded "Il semble donc que de gland thyroide ne joue aucune rôle dans le phénomene de l'anaphylaxie. "What his protocols actually showed was

(1) There were 2 control animals, one of which lived.

(2) The shocking dose of antigen was given mainly intraperitoneally and in 3 cc. amounts.

  (a) Animals were sensitized 2, 7 and 11 days after operation. Of each set, 2 died after marked delay after the intraperitoneal injection of 3 cc. of antigen. Two died 5 minutes after the intracardial injection of 1 cc. of antigen. The animal sensitized the seventh day after operation was sacrificed and thyroid tissue found.

  (b) 2 animals were sensitized at the time of operation, both of which died when given the shocking dose.

  (c) 4 animals were thyroidectomized 2, 2, 7 and 8 days respectively after sensitization and 2 of them lived when shocked.

Certainly this is not a convincing piece of work.

Këpinow and Lanzanberg, in 1923, claimed that Appelmans' contradictory findings were due to differences in technique.

Houssay and Sordelli, in 1923, sensitized 10 guinea pigs with 0.01 cc. of horse serum subcutaneously, 9, 11 and 16 days after thyroidectomy, as well as an unmentioned number of control animals. Ten had a thyroidectomy 6 days after sensitization. The shocking dose of antigen was 2 cc. given intravenously, Whether this was diluted or not was not mentioned. One of the animals operated on before sensitization had a slight shock, the other 9 none, while 66.6 per cent of the controls and 66.6 per cent of the animals operated upon after sensitization died. If 2 cc. of serum was not enough to kill all control animals when given intravenously, if correctly administered, there was either something wrong with the serum or 0.01 was not enough to sensitize, which is improbable. Nevertheless, these are experiments which confirm the work of Lanzenberg and Képinow. No appreciable number of rabbits or dogs could be protected by thyroidectomy.

Parhon and Ballif, in 1923, sensitized with 0.02 cc. of sheep serum 2 thyroidectomized animals 11 days after operation. Twenty days later these animals and 5 controls received 0.1 cc. of shocking antigen intravenously. All of the animals were severely shocked, the two treated animals and 1 control surviving. Due to the small number of treated animals and the fact that they were so severely shocked, one cannot draw definite conclusions.

Appelmans, later in 1923, repeated his work. Animals were sensitized 15 days after operation and shocked intraperitoneally 15 days later. No protocols of any kind were given. He said there was no difference between the operated and non-operated animals.

Parkon, and Ballif, in 1923, also repeated their experiments. A shocking dose of 0.1 cc. killed 7 out of 8 treated animals and 3 controls. A shocking dose of 0.03 cc. killed 1 control and strongly shocked, without killing, 1 treated guinea pig. The authors did not believe thyroidectomy was a help. It is very probable that their minimum lethal dose was incorrect.

Savini, in 1923, in an article without details, mentions further experiments and discusses the protection against anaphylaxis present in animals with too much or too little thyroid.

Nechkovitch, in 1924, sensitized rabbits after thyroidectomy. He found very little difference between his treated and control animals.

Fleischer and Wilhelmj, in 1927, in a well controlled experiment, corroborated the work of Lanzenberg and Képinow to the effect that 26 out of 31 control animals died, whereas only 20 out of 53 animals died which had been thyroidectomized, 11 to 30 days before sensitization. The amount of the shocking dose was not given. They were not able to agree with the French writers that the thyroidectomized, sensitized animals were unable to produce passive sensitization. Their table showed that there may have been a slight diminution of this power but certainly very little. In this way they agree with Houssay and Sordelli. *Fleischer and Wilhelmj* (1927) correlated their findings with surface tension changes of plasma.

Summary. From the evidence at hand, it is shown that the effect of thyroidectomy before sensitization is to change in some way the animal's ability to react strongly to the same amount of shocking antigen that kills the control animals.

Thyroidectomy after sensitization seems to have no effect. Further work must be done to show whether thyroidectomy will markedly reduce the ability of a properly sensitized animal, as Lanzenberger and Képinow believe, to produce passive sensitization in a normal animal. That is, it should again be shown that sera from sensitized animals, previously thyroidectomized, will not confer passive sensitization on normal animals.

Thyroid extract

Savini and Savini, in 1915, fed sensitized guinea pigs extract of thyroid for two days before they received intravenously the shocking dose of 1 cc. of horse serum. An unstated number of controls died and an unstated number of treated animals lived. The authors made an interesting observation that if rabbits used for the production of haemolysins, were fed thyroid before the antigen, there were very few accidents in contrast to the animals not given thyroid.

The work of *Kêpinow* (1922), in which thyroidectomized guinea pigs were given thyroid extract has been referred to under Thyroidectomy.

Kêpinow, in 1923, concluded that weak doses of thyroid gland per os the day of shock were of no value, the same doses attenuating shock if given 2 days before. Larger doses of thyroid gland extract, however, augmented shock. The experiments were controlled and seem valid, although the number of animals used for each variation of treatment was small. The author believed that the action of the thyroid influenced the formation of anaphylactic antibodies.

Toxins

Billard and Barbès, in 1913, stated without experimental evidence that poisons selective for the nervous system, such as venoms, tetanus toxin, etc., could attenuate anaphylactic shock.

Transfusion

Manwaring, in 1910, found that sensitized dogs, bled and given 1200 cc. of normal dog blood, still reacted to the shocking dose of antigen.

Trikresol

Rosenau and Anderson, in 1906, obtained no inhibition of shock by adding 0.4 per cent of trikresol to the second dose of serum. This was used with ether as a preservative of the serum.

Trypan blue

Isaacs, in 1925, gave trypan blue to guinea pigs 10 days before sensitization and during the sensitization period without effect on shock. He therefore concluded that a different mechanism was involved in the production of anaphylactic sensitization than in the formation of anti-sheep hemolysis, as experiments in the production of haemolysin had shown that it was reduced in dye treated animals. No protocols were given.

Lewis and Loomis, in 1926, concluded that the allergic irritability of the guinea pig, that is, its capacity to react to antigenic substances, was increased by treatment with trypan blue. The technic employed renders the experiments non-comparable to others.

Klinge, in 1927, studying local anaphylaxis, found that inflammation could be lessened or entirely prevented by the injection intravenously or directly into the tissues of trypan blue the day before sensitization or just before the second injection of antigen. Protocols were not given in the article, which was a short summary of the work done.

Summary. There is no conclusive evidence that anaphylactic shock can be modified by treatment with trypan blue.

Ultra violet light

Giaume, in 1928, concluded that treatment of the serum used for the second injection of antigen with ultra violet light resulted in an inhibition of shock in animals with normal serum, not in those sensitized with irradiated serum. In 1 set the 6 controls died, while 12 animals had no shock that received serum which had been irradiated with a Bach lamp of 110 volts, continuous current, 1500 C.P., at a distance of 50 cms., for 30 minutes. Serum was treated from 30 to 60 minutes before use. In another series, 4 controls died and of 4 animals which had been given serum irradiated for 5 minutes, 3 survived moderate shock and 1 died of hemorrhage. Four animals given serum which had been treated for 30 minutes had no shock.

Urethane

Besredka, in 1908, found that 0.4 gram of urethane prolonged the life of shocked guinea pigs. No protocols were given and the number of controls was not stated.

Anderson and Schultz, in 1909, as has been discussed under "Adrenalin" gave combined treatments of urethane, chloralhydrate and adrenaline and also in some cases, artificial respiration with oxygen. The rôle of the urethane in these experiments cannot be determined, but the results with the treated animals were better than with the controls.

Rosenau and Anderson, in 1909, found no definite inhibition of shock by giving sensitized animals 0.9 or 1.0 gram or urethane by mouth 45 or 70 minutes before the intraperitoneal injection of 6 cc. of the antigen.

Urine

Pfeiffer, in 1911, studying the increased toxicity of urine in anaphylactic shock, carried out a few experiments on the inhibition of shock with urine. In one series (Table 33, page 611) guinea pigs were sensitized with normal horse serum and given 2 intraperitoneal injections of toxic, anaphylactic urine, 18 and 19 days after sensitization. The second injection of antigen was given 48 hours after the second treatment. As compared with the 3 controls, the 2 treated animals were definitely protected.

In a similar series (Table 34, page 642), sensitized guinea pigs were given 2 intraperitoneal injections, as in the first series, of 2 cc. of toxic urine and it was found that 5 of the 8 treated animals were protected, as compared with the 8 untreated controls. No experiments with normal urine were described. We therefore have some evidence of the protective effect of urine, but none as to whether this is limited to anaphylactic urine or not.

Vagus section

Auer, in 1910, was unable to find that section of the vagus before or after desensitization had any effect upon the production of anaphylactic shock. Neither did complete or partial degeneration of the vagus nerve.

Auer and Lewis, in 1910, found that section of both vagi had no protective effect upon shock, such as would be expected if the action of the second injection of antigen was upon the medullary centers. There were no protocols for this work.

Friedberger and Gröber, in 1913, believed that they obtained definite protection against 3 lethal doses of antigen by vagotomy. Of the 8 untreated controls, 7 died after from 0.23 to 0.35 cc. per kilogram of the serum antigen and the 8th animal survived 0.26 cc. per kilogram with shock. Of the 4 guinea pigs with section of the vagus, 2 had bilateral section. One of these died 15 minutes after 3.7 lethal doses of antigen, the lungs on autopsy being a little distended. The other had light shock. One animal had left vagotomy and died without distension of the lungs 25 minutes after 4 lethal doses of antigen. The animal which had right vagotomy had no symptoms from 3.5 lethal doses.

Galambos, in 1913, continued the work of Friedberger and Grober. He found that of 3 guinea pigs with both vagi cut, 1 died of anaphylaxis after 0.6 cc. of antigen, 1 which had 0.4 cc. of antigen, was ill, but recovered and another died 30 minutes after 0.2 cc. of antigen, with no anaphylactic pathology. Of two animals with the right vagus cut, 1 survived 0.2 cc. of antigen and the other died from 0.6 cc. Of the the controls, 3 untreated animals were killed by 0.12 cc. of antigen.

Summary. Work has been done which shows that vagus section may, in some cases, prevent fatal anaphylactic shock.

Vitamine A deficiency

Wedgewood and Grant, in 1924, working with rats, concluded that vitamine A deficiency did not render their animals more sensitive.

Vitamin B deficiency

Aberhalden and Wertheimer, in 1922, sensitized pigeons with beef serum and kept them on a diet exclusively of polished rice. The second injection of antigen was given upon the appearance of alimentary dystrophy, that is, in about 20 days. The 4 dieted animals had much more shock than the controls. The technique employed was that of observing temperature changes. All of the treated animals died, but only 1 of the controls.

Wedgewood and Grant, in 1924, found that if young rats were given a diet deficient in vitamin B, they were predisposed to sensitization, or the effect of sensitization was increased. Five rats were kept on a vitamin B deficient diet for 2 weeks or longer during the sensitization period, and died when shocked. The authors concluded that vitamin B—either desensitized, prevented sensitization or protected against shock.

Vitamin C

Zolog, in 1924, kept guinea pigs on a scorbutic diet for 1 month before sensitization and during sensitization. He then found them resistant to 8 times the dose of antigen lethal for undieted controls. This dosage of antigen was calculated per 100 grams of weight.

Wedgewood and Grant, in 1924, concluded that vitamin C deficiency did not render young rats susceptible to sensitization.

Wedgewood, in 1924, kept 5 guinea pigs on a complete diet during 2 weeks of sensitization and found that then 1 cc. of a 10 per cent solution of the antigen, egg white, given intraperitoneally, killed all of them. In the treated series, 5 guinea pigs were given intraperitoneally 5 cc. of sterile, neutralized orange juice daily during sensitization. These animals had shock but recovered when given the same amount of antigen as the controls. In another group, kept on a scorbutic diet for 16 days before shock, 4 died and 1 recovered from shock.

Water

See also Mineral Waters.

Besredka, in 1907-c, found that precipitation of the serum antigen with distilled water did not diminish the toxicity.

Richet, Brodin and Saint-Girons, in 1919, showed that in 3 dogs, no protection against shock was obtained by diluting the shocking dose of antigenic serum with 9 times its volume of distilled water. The amount of antigen may have been too high to allow the determination of small differences.

Whey protein

Besredka, in 1908, found that while whey did not sensitize, when it was given intraperitoneally to guinea pigs which had been sensitized with milk, the animals were protected against the second injection of milk, given intracranially. He produced anti-anaphylaxis with 10 cc. of "lait cru," given by rectum or by mouth. The number of controls was not stated, 0.1 cc. of antigen being the lethal dose.

Banzhaf and Steinhardt, in 1910, believed that Besredka had diluted the active protein, not separated it into fractions.

Bauer, in 1912, sensitized animals with 1 cc. of whole milk, given intraperitoneally and treated them with from 0.5 to 10 cc. of whey protein, given intravenously. One animal was used for each dosage. For shock, 4 cc. of casein was given intravenously 1 day after the whey protein injection. One of the 2 controls which received 4 cc. of casein intravenously recovered after severe shock, and the other died. Of the 5 animals treated with different doses of whey protein only the animals receiving the smallest amount of treatment, 0.5 cc. died. The 2 which had 1.0 and 2.5 cc. of whey had severe or moderate shock but recovered. Those which had 5 and 10 cc. of whey had mild or no shock. In another series of experiments, Bauer found that the 5 animals, treated with 0.5 to 20 cc. of whey protein intraperitoneally the day before shock, had mild shock upon the injection of 1 cc. of casein intravenously. The 3 controls died after the same amount of antigen. This second series, which was more convincing than the first, because better controlled, seems to offer definite evidence that whey protein inhibits anaphylactic shock with casein in animals sensitized to milk.

Reviews. In addition to these articles on particular aspects of this problem, the following reviews on the inhibition of anaphylactic shock may be cited. Thomsen, 1917, Kopaczewski, 1920, Widal, Abrami and Vallery-Radot, in 1921, who gave no bibliography, and the short paper by Isaacs in 1929. The problem is also discussed briefly in the more general reviews on anaphylaxis, notably those of Doerr, in 1913, of Kraus, in 1919, of Coca in Tice's Practice of Medicine, of Wells, in 1921, of Doerr, in 1922, of Dale, in 1922, and of Longcope, in 1923.

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THE CONTROL OF TONUS AFTER INJURIES TO THE BRAIN OR SPINAL CORD OF MAN

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Some years ago the author published a review dealing with the morphology and physiology of the postural reflex (Langworthy, 1928). By the postural reflex is meant the precise control of the pattern of tonus that makes it possible for the organism to counteract the force of gravity and to balance upon the legs. In this review experimental data concerning the physiology of the postural reflex was presented and it was shown that this informaton was conflicting at almost every point. One very illuminating result of this experimental work, however, makes the whole subject more comprehensible. Tonus is not controlled or mediated by one part or another of the nervous system but is a function of the entire brain and cord acting together, a function of the total individual (Cobb, 1925). Under these conditions it is difficult to evaluate the part played by different structures in the brain in this perfect control. Yet this is exactly the thing that the physiologist has wished to do. The question always arises in physiology as to the way in which results obtained from study of the dismembered organism correspond to the pattern as seen in the intact individual.

Walshe (1929) has recently emphasized the fact that great care must be used in studying a problem such as tonus from the standpoint of phylogeny and applying the results obtained in the experimental animal to the problem of tonus in man. This has also been pointed out by the present author when he suggested, for example, that Rademaker's findings in the rabbit did not apply in their entirety even to other laboratory mammals.

It must be clearly understood that, in the course of phylogenetic development, now one portion of the central nervous system and now another has gained a dominant control over the tonic reflex. From time to time under the stimulus of increased peripheral or central connections, portions of the brain have undergone enormous increase in size and from these areas new efferent pathways have developed, discharging their impulses either directly or indirectly upon the cells of the final common motor path. In phylogeny now one efferent pathway and now another has assumed a dominant control over the tonic and phasic contractions of striated muscle. In lower vetebrates, local reflex arcs in the spinal cord are capable of controlling the postural reflex in a fairly normal manner without the aid of impulses from the brain. But in the ascending phylogenetic scale, the final common path has come to be more and more under the domination of higher cerebral centres. The first important efferent pathway from the brain arose from scattered cells in the reticular formation of the brain stem; the vestibulo-spinal and then the rubro-spinal traits were developed later. The efferent connections of the corpora striata show important phylogenetic variations as Wilson (1914) has pointed out. But in mammals these nuclei make only indirect connections with the final common path by way of the red nucleus, substantia nigra and thalamus. Finally, the cerebral motor centres have undergone progressive differentiation, exerting their influence directly or through the cerebellum. Studies of the postural reflex, then, must take into consideration the relative functional importance of these efferent pathways in the particular animal studied experimentally. Among many well-known examples of the importance of this principle one might mention the wide variations in the behavior of man compared with the common laboratory mammals after injury to the cortico-efferent tracts or after complete transection of the spinal cord.

Since there exist these differences in the functional importance of cerebral centres in different mammals it seems important to gauge their relative importance in one animal. An opportunity to study the pathways controlling tonus in man is made possible by the recent investigations particularly of English clinical neurologists. There are, for example, the observations of Riddoch on spinal man (1917), of Holmes on cerebellar injuries (1917 and 1918), of Wilson (1914) on disorders of the basal ganglia and Walshe's (1929) interpretation of hemiplegia on the basis of modern physiology.

In order to correlate this material, tonus will be considered in the present paper under the following headings: the control of tonus through a local reflex arc in a single segment of the cord, control by intersegmental spinal reflexes, by the brain stem, by the cerebellum, by the corpus striatum and by the cerebral cortex. Although it would be best to consider each of these portions of the nervous system in terms of reflex arcs, controlling the primary reflex arc through the muscle, it is extremely helpful in this general discussion to begin by presenting Sherrington's thesis of the final common path.

It must, of course, not be forgotten that it is idle to speak of motor pathways and influences mediated by them without keeping the idea of the reflex arc clearly in mind. The motor responses are in every case governed by sensory stimuli which activate the motor side of the reflex pathway. Inasmuch as the sensory and correlation pathways are so difficult to study anatomically and physiologically, it is perhaps permissible to simplify this extremely difficult problem of tonus as much as possible and focus attention on the motor side of the reflex pathway. This has ever been the tendency of clinical and non-clinical investigators.

THE FINAL COMMON PATH

Sherrington (1906) suggested that all the motor cells in the anterior gray columns of the spinal cord and all the motor cells of the cranial nerve nuclei be grouped together and called the final common path since these neurones form the only pathway by which stimuli from central nervous system may exert an influence upon the tone and contraction of muscle or the activity of the glandular structures of the body. It makes no difference how many impulses enter the nervous system, the only way they can produce a response is through the final common path.

Herrick and Coghill (1915) have given very interesting suggestions concerning the phylogeny of the final common path and have pointed out that in the course of development there has been a marked and progressive development in the motor activity possible. They compare the central nervous system to a funnel in which the sensory impulses are poured in at the top and the response through the final common path forms the outlet. In amphibia, for example, large numbers and types of sensory responses enter the nervous system but the final common path is little differentiated; the one response of a tadpole to nocuous stimulation consists in swimming. In higher forms the sensory stimuli entering the nervous system are perhaps no more numerous or diversified but the possible motor responses have increased enormously in number.

The final common path consists obviously both of somatic motor and autonomic cells. Several years ago it was suggested that the tonus of striated musculature was mediated through the autonomic nervous system. Many further studies have shown that this is not true: indeed it is doubtful whether autonomic fibers end upon striated muscle fibers (Hines and Tower, 1928). It, therefore, is clear that the somatic motor neurones control both the tone and contraction of the striated musculature of the body.

It is possible to group in a rough way the types of impulses that act upon one anterior horn cell in the cord that may be taken to typify the final common path. In the first place it receives influences from sensory fibers entering the same segment of the cord in which it lies. It also receives impulses from other segments of the cord. The pathways from the brain are reticulo-spinal, vestibulo-spinal, medial longitudinal fasciculus, rubro-spinal, tecto-spinal and cortico-spinal. There may be others but recent work has suggested that even the autonomic component of the final common path is correlated and controlled by impulses through these same well known pathways (Langworthy and Richter, 1930). Perhaps a few suggestions concerning the morphology of these pathways may be given in this place.

1. Reticulo-spinal tract. Throughout the reticular formation of the brain stem from the lower end of the medulla to the upper end of the midbrain are scattered motor cells. These cells give rise to a diffuse pathway which travels down into the spinal cord. The pathway is old phylogenetically and probably was the first pathway from the brain to influence the anterior horn cells. It is still probably tremendously important from the standpoint of tone for it is very likely that many of the efferent impulses from the corpus striatum and cerebellum are relayed along this route. The fibers are both crossed and uncrossed.

2. Vestibulo-spinal tract. The vestibulo-spinal is also an old pathway from the standpoint of phylogeny and an extremely prominent one in controlling the pattern of tonus in connection with vestibular influences. It arises from the cells of a distinct portion of the vestibular nucleus called Deiter's nucleus. Around these cells end the primary vestibular neurones. The cells, however, do not have the structure of other sensory neurones of the second order but resemble motor cells. The vestibulo-spinal pathway is an uncrossed one. Vestibular fibers also cross in the midline and run caudally in the medial longitudinal fasciculus.

3. Medial longitudinal fasciculus. The medial longitudinal fasciculus is a complex pathway consisting of many groups of fibers (Langworthy, 1929). It has been mentioned that fibers from cells in the vestibular nucleus cross in the midline and travel caudal to influence the cells of the final common path. Vestibular fibers also run in a cephalic direction and place the cells of the extraocular nuclei under vestibular influence. It also contains fibers that correlate the activity of all the cranial nuclei in the brain stem.

4. Rubro-spinal tract. The rubro-spinal tract arises from the large cells at the caudal end of the red nucleus. It is said to be relatively small in man. The fibers cross the midline. The red nucleus receives fibers from the opposite hemisphere of the cerebellum and from the globus pallidus, the place of origin of the efferent pathway from the corpus striatum. Its part in the control of tonic patterns is great but never has been adequately defined.

The red nucleus in lower mammals consists in the main of the group of large motor cells giving rise to the rubro-spinal tract. In higher forms a group of small cells have developed cephalic to the large cells. These cells give rise to axones which end around the cells of the reticular formation. It has been suggested that the large-celled portion received impulses from nuclei globosus and emboliformis of the cerebellum; the small-celled portion from the dentate nucleus. In man the small-celled portion is very large and the large-celled portion at least relatively diminished in size. Since the small cells give rise to fibers ending around the motor cells of the reticular formation, their influence upon the cells of the final common path utilizes an indirect pathway, the morphology of which is not clearly understood.

5. Tecto-spinal tract. The tecto-spinal tract arises from large cells in the deeper cell layers of the optic colliculi. The fibers cross in the midline and run caudally just ventral to the medial longitudinal fasciculi and lateral to the raphe, to influence the cells of the final common path. These cells must influence the pattern of tonus in relation to stimuli from the visual apparatus.

6. Cortico-spinal tract. The cortico-spinal or pyramidal tract is of great importance in man. The giant motor cells or Betz cells of the anterior central gyrus give rise to the fibers. This pathway is, in general, the agency for the production of volitional quick oscillating movements of the extremities.

7. Fronto-pontine and temporo-pontine tracts. It is believed that the fronto-pontine pathway arises from cells just anterior to the dorsal portion of the anterior central area. The exact region from which the temporo-pontine pathway arises is not yet clearly understood. These pathways end around cells in the pontine nuclei and exert their influence through the cerebellar hemispheres. The fronto-pontine and temporo-pontine fibers probably exert a normal inhibitory effect over muscle tonus and inhibit the tonus of the opposing muscles when the cortico-spinal pathway initiates precise quick movements. The physiology of these pathways from the cerebral cortex will be discussed in detail in a later portion of this paper.

THE CONTROL OF TONUS BY AN INDIVIDUAL SEGMENT OF THE CORD

With this hypothesis of the final common path and the impulses which play upon it clearly in mind the question of tonus must be considered in terms of reflex arcs involving different portions of the nervous system. The attention however may always be focused on the anterior horn cells since they represent the final portion of each of the reflex arcs.

Naturally there is little information concerning the physiology of the individual spinal segment in man and the data here are experimental. Sherrington showed that the tonus of the striated musculature was primarily dependent upon sensory impulses from the muscles themselves. The proprioceptive fiber from the muscle enters the cord through the posterior root and immediately establishes reflex connections with the anterior horn cell. The adequate stimulus for the sensory endings in the muscle consists of stretching; in other words, tonus is fundamentally a stretch reflex.

Sensory impulses from the cutaneous surface of the segment have very little influence upon the tonic reflex. It was shown however by Sherrington (1906) that stimulation of nerve trunks, such as the sciatic, produced either augmentation or inhibition of the tonus. Denny-Brown (1929) has suggested that the two types of sensory endings in the musculature, the tendinous endings and neuromuscular spindles, influence tonus in different ways. When the muscle is stretched, the neuro-tendinous endings are first stimulated and send impulses into the segment that increase the tonus. If the stretch is continued the neuromuscular spindles that require a higher threshold stimulus influence the activity of the cord in the direction of inhibition. If this is true it is an important explanation of certain of the qualities of tonus in the hemiplegic which will be discussed later.

Walshe (1929) has been able to show that the hypertonus present in the musculature of man under pathological conditions is also dependent on influences from the proprioceptive endings in the muscle. If the proper amount of one per cent novocaine is injected into a hypertonic muscle the sensory nerve endings may be paralyzed and the motor endings unaffected. Under these conditions the increased tonus of the muscle is lost but voluntary power retained.

If tonus is thought of as controlled by an isolated segment of the cord, the tonus is a property of both the flexor and extensor muscles. With certain exceptions that may be disregarded, tone is not a pattern at this level but a property of all striated musculature dependent on afferent impulses from the muscles themselves acting on the anterior horn cells whose axones influence the muscles. When several segments of the cord are acting together and many impulses are influencing the anterior horn cell, tone becomes correlated into a pattern.

ABNORMALITIES OF TONUS AFTER COMPLETE TRANSECTION OF THE SPINAL CORD OF MAN

It is important to consider further the control of tonus by the isolated spinal cord of man after all influences from the brain are cut off. The careful war-time observations of Riddoch (1917) make this possible. He studied a number of previously healthy young adults in whom the spinal cord was completely transected in the upper thoracic region. For the first few days following the injury no superficial or deep reflexes could be elicited from the portion of the body innervated by the isolated cord. The skin was dry. The bladder became distended and the patients had to be catheterized.

After a variable period of time, not more than seven days in some cases, reflex activity returned. The reflexes in favorable cases became much more active than normal so that a slight cutaneous stimulation of the footpad produced a marked contraction of the flexors of both legs and of the musculature of the ventral body wall. The bladder was evacuated at the same time and there was marked sweating over the whole area of skin innervated below the lesion. Riddoch demonstrated that this was a mass reflex; the local reflex arcs lost their local sign and a sensory stimulus excited all the anterior horn cells to activity. It suggested that cerebral control of the anterior horn cells, both somatic motor and autonomic, is predominantly an inhibitory one.

On more careful examination it is quite evident that not all the somatic musculature innervated by the isolated segment took part in the mass reflex. The muscles that contracted strongly were always the flexor muscles; indeed if any extensor reflexes were present in the legs, Riddoch was able to hypothesize that the cord was not completely severed.

The response of the musculature in these cases followed a very definite pattern. After a nocuous stimulus was applied to the plantar surface of one foot, both legs were sharply flexed and the abdominal musculature also contracted. It has been suggested that this is the pattern of a primitive response to painful stimulation, withdrawing the legs from danger and protecting the vulnerable abdominal wall from injury.

Babinski phenomenon. Certainly this mass reflex is but an accentuation of the Babinski phenomenon which is elicited from the legs after injury of the cortico-spinal tracts. The Babinski reflex is commonly thought of as a dorso-flexion of the great toe and fanning of the other toes on stimulation of the outer plantar surface of the foot. When well developed, however, it is a general flexor response involving the ankle, knee and thigh. Although the locus of the sensory area is on the outer plantar surface of the foot, a response may be obtained on painful stimulation over any portion of the leg or thigh as high as the inguinal region. The response obtained in spinal man differs from the Babinski only in a degree.

Paraplegia in extension. There is another clinical picture that must be considered with a discussion of the control of tonus by the isolated spinal cord. If a tumor growing outside of the cord begins to press upon the spinal cord in the upper thoracic region, the pyramidal tracts, which appear to be particularly vulnerable to this type of injury, early become functionless. This releases a tonic pattern controlled in the region of the brain stem from its normal inhibitory control and a hypertonus is present in the extensor musculature of the legs. Loss of the cortico-spinal control also produces a relative paralysis in the flexor muscles of the legs. The legs are strongly abducted and extended. The Babinski reflex may be elicited bilaterally and the abdominal reflexes are absent from the portion of the abdominal wall innervated below the injury. Clonus may be demonstrated in the legs. This syndrome is known as paraplegia in extension.

Paraplegia in flexion. If the tumor, however, continues to grow and press upon the cord until a physiological transection of the whole cord is produced, the pattern of tonus is quite changed until it is similar to that found in man after complete transection of the spinal cord. The hypertonus of the extensor musculature is lost and no reflexes involving the extensor muscles can be obtained. The tonus is now increased in the flexor muscles so that the legs are flexed at hip and knee. Moreover, painful stimulation produces strong contraction of all the flexors innervated below the level of the lesion. This is known as paraplegia in flexion.

With these data it is possible to discuss the control of tonus by the isolated spinal cord. In the case of paraplegia in extension the cortico-spinal tracts are functionless but other pathways connecting the brain and spinal cord are still normal. The loss of the cortico-spinal control releases tonic centers in the brain stem producing a picture of partial decerebration which will be discussed at some length under the heading of hemiplegia and injuries of the cortico-spinal pathways.

The abnormalities in tonus after complete transection of the cord and in cases of complete physiological transection due to pressure are identical. Riddoch observed that in spinal man tonus always returned, first in the flexor muscles, then in the extensors and was always more marked in the flexors.

The period of a-reflexia following the cord transection is due to the removal of efferent stimuli from the brain to the anterior horn cells. The cells of the final common path are so dependent on this cerebral innervator that for a time the stimuli reaching the cells from spinal reflex arcs are not sufficient to produce reflex activity. When the local mechanism does become self sufficient it overacts to a marked degree since inhibition is the predominant cerebral influence upon the anterior horn cells.

The anterior horn cells innervating the extensor muscles are more under the influence of centres in the brain than are the cells innervating the flexor muscles. In general it may be said that the flexor muscles produce the quick oscillatory movements; the extensor muscles contract the force of gravity. The quick movements may be controlled by the spinal cord whereas that pattern of tone which has to do with the antigravity reflex is predominantly controlled by centres in the brain stem. Since the tonus of the flexor muscles is less under the control of centres in the brain it recovers more quickly and completely in the isolated cord.

Further references to experimental work will make this thesis clearer. The author has previously suggested (1928) that the act of walking may be thought of as composed of two factors. A tonic contraction of the extensor muscular counteracts the force of gravity and supports the body from the ground, while alternating rhythmic flexor and extensor movements of the extremities produce a change of position and are responsible for locomotion. Stepping is a fundamental activity of the nervous system,—rhythmic in nature and dependent on two peripheral, antagonistic forces, one tending toward flexion, the other toward extension. Now one force, now the other becomes dominant, the antagonistic action being successively in a state of inhibition. This tendency to rhythmic activity is, as shown by the experiments of Sherrington, a property of the isolated spinal cord. The proprioceptive fibers from the muscles carry the afferent impulses responsible for reflex stepping. Graham Brown (1912) demonstrated that even the integrity of the proprioceptive pathway was not necessary. The rhythmic tendency may be maintained on the motor side of the reflex arc and under certain conditions, such as asphyxia, these motor centres may be influenced to rhythmic activity by substances contained in the blood stream after the manner of the respiratory center.

The antigravity reflex on the other hand, is controlled by centres in the brain stem rather than the spinal cord. In man the extensor muscles of the legs, trunk and neck preserve the body in the erect position. It has already been observed that after transection of the cord in the thoracic region that tonus is most marked in the flexor muscles and the reflexes that may be elicited are flexor reflexes.

There is more of the ability to stand perhaps in the case of laboratory mammals with the thoracic cord transected than in man. After a cat has recovered from the shock and the posterior portion of the body is reflexly active it is able to support its weight on the legs for several seconds if the hind-legs are abducted widely and placed as props. There is no ability, however, for the tonus to accomodate itself to new positions and the hind-legs soon give way, even if the cat stands quietly.

It may be mentioned that Foerster, in Germany, has observed some extensor responses in human patients after complete transection of the spinal cord, verified at operation. Thus a crossed extensor reflex could be demonstrated in the legs. The extensor responses do not appear until a period of years following the accident and he believes that the English investigators did not follow their cases long enough to observe them.

In summary, quick, oscillatory flexor and extensor movements are a property of local reflex mechanisms in the spinal cord; the patterns of tonus regulating the postural reflex and involving the extensor musculature are controlled by centres in the brain stem. The anterior horn cells controlling flexor tonus are less under the control of centres in the brain and recover more fully than the cells controlling extensor tonus when the spinal cord is made to function alone.

MECHANISMS IN THE CORD CONTROLLING PATTERNS OF TONUS

Thus far only the activity of the lower portion of the cord after a transverse lesion in the thoracic region, has been discussed. No cases of survival after transection of the cord in the upper cervical region in man occur. This lesion isolates the muscles of respiration from the respiratory centre or injures the phrenic nuclei directly. The activity of the isolated cord after transection at the level of the first cervical segment would no doubt be somewhat different from that in the thoracic region since more intersegmental influences would act upon the cells of the final common path to correlate the pattern of the tonus in the musculature.

There is, in the upper two segments of the cord, a centre having a marked influence upon tonus in the extremities. There the regulation of tonus has to do with proprioceptive influences from the neck musculature which regulate the tonus pattern in reference to movements of the head.

Tone in the striated musculature of the body must be thought of continually as a delicate and ever changing pattern which is constantly varying in a normal individual. The afferent stimuli that have the most influence in varying the pattern of tonus are precisely those which become maximal when the position of the body is changed. Thus the vestibular stimuli are of great importance. So also are impulses arising in the neck muscles. This is very easy to understand when it is realized that any change in the position of the head requires an instant shift in the tonic pattern in all the striated musculature of the body to preserve balance.

Tonic neck reflexes. It is difficult to evaluate the relative importance of the different types of afferent impulses upon the pattern of tonus except in the experimental preparation. Magnus (1924) decerebrated cats in which the labyrinths had previously been destroyed bilaterally. He was then able to show that rotation of the head around the long axis of the body produced an increase in tonus in the legs toward which the jaw was facing and a decrease in tonus on the side toward which the occiput was facing. Strong flexion of the neck produced a decrease in extensor tonus of the musculature of the fore-legs and an increase in tonus in the hind-legs. It was as if the animal were looking under a shelf. Conversely extension of the neck produced an increase in extensor tonus in the fore-legs and a decrease in the hind. The cat was then looking up on a shelf. In these preparations common tonic patterns, operating constantly throughout the life of the individual, were isolated and accentuated for demonstration. It has been pointed out that these neck reflexes may be utilized in bringing powerful animals into a lateral or supine position. Thus at the rodeo the cowboy is able to force the strongest steer on its side or back by passive movements of the animal's head.

Hemiplegia produces in man an accentuation of tone in certain muscles of the extremities that may well serve as an adequate background for demonstration of the neck reflexes. They occasionally may be brought out in these patients but are seen clearly in only a small number of subjects and the patterns are more complex and less predictable than in animals. This is partly due to the fact that the spasticity in the arms is present in the flexor muscles rather than in the extensors as in four footed animals. A discussion of the problem of patterns of tonic neck reflexes in man may be deferred till a later part of this paper where the whole question of hemiplegia is considered.

The afferent impulses from the neck musculature must be conveyed by pathways to the somatic motor cells of the entire cord. The effect of these stimuli is to increase the tonus in certain groups of muscles and decrease the tonus in others. These impulses act upon a segmental reflex already described which is primarily responsible for muscle tone but by augmenting or decreasing the activity of this primary reflex are able to produce correlated tonic patterns. It certainly is not possible to say that tone in general is increased or decreased by the activity of these influences.

MECHANISMS IN THE BRAIN STEM CONTROLLING PATTERNS OF TONE

1. *The vestibular apparatus*

It is generally agreed at the present time by all investigators that the fundamental mechanisms for the production of tonic patterns lie in the mid-brain and medulla. The anatomy and physiology of these units are far from clearly understood. Certain statements concerning the comparative development of the brain stem offer an explanation for the development of control of tonus in this region.

The vestibular nerve and its connections in the brain stem appeared early phylogenetically, and assumed a dominating control over a former segmental organism. In the case of the lowest vertebrates there is very little development of a brain and the segments of the cord are coördinated largely by intersegmental neurones. The evolution of the vestibular nerve in higher forms introduced a new non-segmental mechanism for the coördination of tonic activity. With further differentiation of organisms a further cephalization of nervous function has continually taken place and the control of patterns of tonus has shifted from the region of the spinal cord and into the brain stem, particularly into the region dominated by the vestibular nerve.

Since it is now generally believed that embryological development closely follows phylogenetic, the early differentiation of the vestibular mechanism in the human embryo assumes importance. In the early human embryo, rapid growth of the nervous system first occurs in the region of the brain stem and this structure at one time has great relative size. At first it is a simple tube, but eminences soon appear upon the surface due to rapid cell proliferation in localized areas. The first areas undergoing this rapid growth are vestibular and the vestibular nuclei may be identified in all embryos over two somites. In a similar manner the vestibulo-spinal tract and the medial longitudinal fasciculus are the first long pathways connecting the brain and the cord that receive their myelin sheaths.

The importance of the vestibular centres in the production of tonic patterns was early demonstrated by experimental physiologists. If the brain stem of the cat, for example, is completely transected at the level of the mid-brain an accentuation of tone appears in the extensor musculature of the body; the phenomenon is called decerebrate rigidity. If the transverse section be made caudal to the vestibuli nuclei, severing their connections with the cord, tonus is completely lost in musculature. It is clear, then, that transection of the brain stem at the level of the mid-brain frees the tonic mechanism from normal inhibitory control; that transection caudal to the vestibular nuclei abolishes the tonic patterns and produces a complete atonia of the musculature.

The fundamental influences for the production of tonic patterns at the level of the vestibular nuclei are undoubtedly stimuli from the labyrinths although it is probable that other influences, for example, impulses from the cerebellum, reach this region and there is here the development of a correlation centre for tonus. Magnus has studied the tonic labyrinthine reflexes. The tonic neck reflexes were abolished by fixation of the neck of the experimental animal in a rigid plaster cast. The preparation was then held in various positions in space. When the animal was placed in a supine position with the angle of the snout forty-five degrees above the horizontal plane, the extensor tonus in the legs was maximal. The position of minimal extensor tonus was the prone position with the snout at an angle of forty-five degrees below the horizontal plane. With lateral positions of the body between pronation and supination and with variations in the angle of the snout with the horizontal, the extensor tonus was intermediate, being greater or less according to the position approximating the maximum or minimal extensor tonus. There is reason to believe that these reflexes depend on the otolithic apparatus of the utricle, since in the maximum position for these reflexes, the otoliths exert a maximal pull on the otolithic membrane, whereas in the minimal position they press upon the maculae.

These tonic vestibular reflexes are difficult to demonstrate in man, isolated from other factors producing tonic patterns. They have been demonstrated in very young children and in patients with hemiplegia. Undoubtedly tonic reflexes from the labyrinths are important in producing the tonic patterns in man. The exact pattern, however, varies in different mammals.

Two pathways from the vestibular nuclei carry impulses to the spinal cord as has already been explained. The large cells of the lateral, or Deiter's nucleus give rise to an uncrossed vestibulo-spinal path while axones from cells in the descending nucleus cross in the mid-line and run caudal in the medial longitudinal fasciculus. It has been suggested that the uncrossed pathway is excitatory, the crossed inhibitory.

The previous discussion has suggested that the vestibular nuclei relay impulses to the spinal cord controlling tonus in relation to vestibular and other influences that reach these cells. This tonus control regulates a unified pattern which can be varied by changing the position of the head. The question then arises as to whether these impulses may be said to increase or to decrease the tonus of the musculature as a whole. Although this possibility scarcely fits in with the idea of shifting patterns of tonus it certainly seems to be the case. In the experimental animal, as previously described, transection of the neuraxis at the level of the mid-brain produces a great increase in tonus of the extensor musculature of the body. The explanation is that the apparatus controlling tonus is, in this way, freed from normal inhibitory control. The same result is obtained on any section caudal to the mid-brain to the upper end of the vestibular nuclei. If the vestibular nuclei are included in the section and severed in this way from their connections with the spinal cord, all tone in the musculature is lost. There is evidence, therefore, that the influences from the vestibular nuclei in the normal animal reinforce tonus as controlled by the segmental stretch reflex. This becomes important in considering the relief of pathological hypertonus.

2. The mid-brain and the red nucleus

Structures in the mid-brain also have an influence upon patterns of tonus but their exact function has never been clearly understood. Since the red nucleus is the most conspicuous group of cells in this region, to it have been ascribed various functions in connection with the postural reflex. Opinions are very conflicting and only certain views may be introduced here that will help in the interpretation of the pathological syndromes that will be discussed later.

Rademaker (1923) believed that removal of the fore-brain, cephalic to the red nucleus and leaving this structure intact, left the tonus of the musculature and the patterns of tonus entirely normal. Removal of the red nucleus, on the other hand, produced a great increase in tonus in the extensor musculature and the picture of decerebrate rigidity. He thought that, in the common laboratory mammal, tone was regulated in the final analysis, not by the fore-brain but by the red nucleus. The cerebellum according to this observer had no influence upon tonus since no abnormalities followed its removal.

The manner in which Rademaker considered that the red nucleus controlled tonus is important. He felt that sensory impulses from the body musculature, labyrinths and neck muscles were synthesized in the brain-stem to produce patterns of tonus. The labyrinthine and body influences were operative at the level of the red nucleus. These reflexes involving tonus were called "righting" reflexes since they regulated the postures of the animal and tended in every case to bring the animal back into the optimum physiological posture with the body in a prone position and the eyes on a plane horizontal with the ground.

The author has never agreed with these conclusions concerning the rôle of the red nucleus or the phenomenon of the righting reflexes.

There has never been any evidence in Primates, including man, that unilateral or bilateral injury of the red nucleus produces a phenomenon resembling decerebrate rigidity. Moreover transections of the brainstem cephalic to the red nucleus produce abnormalities in tonus similar to those of the decerebrate animal. This whole thesis will be elaborated in later portions of this review. The righting reflexes represent merely the patterns of tonus that are discussed in this paper.

The red nucleus receives influences from the corpus striatum and cerebellum. In order to understand the part played by the red nucleus in the initiation and control of tonus the influence of the cerebellum and corpus striatum must be considered. This will be done in sections which follow.

3. Optic colliculi and optic influences upon tone

It seems clear that optic impulses finally influence the anterior horn cell and produce patterns of tonus regulated by visual stimuli. These reflexes have been studied by several workers and it was thought that they were not important in lower mammals but assumed increasing control in Primates. There is no agreement as to the pathways involved. It would appear probable that this reflex is carried out at a low level in the reflex mechanism and involves the optic end stations in the mid-brain. This seems the more probable inasmuch as there is a pathway from the optic colliculi to the cells of the final common path, the tecto-spinal tract. Experiments, however, suggest that the reflex requires the integrity of the occipital lobe of the cerebral cortex. Following removal of the visual cortex the animal turns the head to the affected side and walks in circles to that side. Later, following the operation, a compensation sets in.

With the rapid differentiation of the cerebral cortex in higher forms it is quite clear as will be demonstrated later, that the control of tonus has been taken over more and more by centres there. The exact pathways required for the optic righting reflexes require further study. Here their great importance in higher mammals may be emphasized.

4. The rôle of the cerebellum in tonic reflexes

Here again the results of animal experimentation are conflicting. Magnus and Rademaker assert that after complete removal of the cerebellum in man, tonus is quite normal in amount and distribution.

Fortunately, in this case, we have direct observations following injuries of the cerebellum of man and those alone need be considered.

Holmes (1917 and 1918) during the war was able to study and correlate the abnormalities following injury of the cerebellum in a large number of cases. He concluded that practicaly all the abnormalities of muscle innervation could be explained on the basis of disturbances of tone. Luciani (1915) had previously summarized the defects following lesions of the cerebellum under three headings, *asthenia* or a weakness and tendency to fatigue, *hypotonia* and *astasia* with staggering gait and intention tremor. All these symptoms may be fundamentally reduced to one underlying cause, the hypotonia.

A summary of Holmes' findings may be discussed at some length. In the first place there was a definite hypotonia in the muscles which was at once apparent to the observer and was illustrated by photographs in his paper. The hypotonia was marked in the first few days and then decreased, but as long as the patient showed any of the changes summarized in the following paragraphs, hypotonia could be said to be present. When the lesion involved one half of the cerebellum the hypotonia was confined to that side of the body. If the lesion was large enough to produce any symptoms at all the whole musculature of that side of the body was involved, not just one limb or one or more groups of muscles. The hypotonia could be demonstrated in a number of different ways. If the hypotonia limb was passively oscillated, its excursions were limited only by the ligaments and joints whereas the excursions of a normally tonic limb are checked to a certain extent by the muscle tone. In severe cases the affected limb was unable to maintain any attitude without support. There was a deficient elasticity of the muscles which could be demonstrated in the arm in the following manner. When a normal arm is suddenly jerked from a position of extension into flexion it tends to spring back into its original position owing to the presence of tone in the extensors of the arm. In the case of the abnormal arm, the forearm either remained in a position of flexion or swayed about uncertainly. This could be demonstrated by another test. The patient was instructed to resist by voluntary contraction the movement of the arm produced by sudden tap. The affected arm showed greater excursion and oscillation than the normal one.

The deep reflexes were also modified by the hypotonia. A light tap, sufficient to evoke a knee jerk in a normal limb, often failed to evoke a response. Also the knee jerk was pendular, undergoing a number of oscillations before it came to rest. These after movements were due to a failure of after-shortening in the quadriceps extensor.

The rebound phenomenon is probably due to a lack of tone in the antagonist muscles. If the patient was told to pull the hand toward his mouth against resistance and the resistance suddenly removed, the normal hand was soon arrested by the action of the antagonists. The affected hand did not stop in this manner and sprang back into the face.

In the absence of normal tone, smooth coördinated movements were impossible because the element of fusion of small contractions was lacking. This accounted for the disturbance of muscular contractions. Fatigability was explained by the fact that in the absence of proper fusion of successive small contractions each contraction shortening the muscles must start at a lower level than that reached by its immediate predecessors and the power of the whole contraction must be less than that of a corresponding normal muscle. Also undue fatigability occurred because the patient relied on voluntary contractions which fatigued without the assistance of tonus which is indefatigable. There was often a delay of initiating contractions and relaxing them. The hypotonic muscles had to take up slack which delayed contraction and relaxation.

There are many demonstrable disorders of movement in cerebellar disease that may be traced to the disorganization of tone. Thus there are errors in the direction, range and rate of movements and a loss of finer coördination of the movements of the fingers. Tremor also occurs due to faulty fixation of the extremity. These abnormalities of movement are well displayed in walking and the characteristic gait is due to a combination of the hypotonia and the efforts of the patient to make corrections for it. The abnormal attitudes and postures probably compensate for loss of tone in the musculature of the abnormal side of the body. Speech is grossly abnormal due to poor coördination of the musculature controlling phonation and articulation.

These examples might be increased and the whole subject expanded further, but this review does not deal primarily with cerebellar disease. Enough evidence has been presented to make it quite clear that abnormalities in tonus underlie most of the abnormal physical signs of cerebellar injuries in man. Moreover, there is always a loss of tone. No symptoms pointing to increase of tone have been observed in man. The cerebellum is built up phylogenetically as a correlation and coördination structure in close connection with the vestibular nerve. One might expect, therefore, that it would control the finer coördination of tone and this seems to be the case. Moreover the evidence presented here indicates that it normally augments the tonic reflexes of the lower reflex centers. Tone as controlled by the cerebellum is a pattern coördinating the finer activity of all the muscles, participating in a movement.

There is some evidence resting on experimental work that certain portions of the cerebellum give rise to impulses that tend to inhibit rather than augment the tonus of striated musculature. For example, stimulation of the cephalic or caudal portion of the vermis produces an immediate and complete loss of extensor tonus in the decerebrate cat. Moreover many observers attest that injuries or total removal of the cerebellum of animals does not produce a picture similar to that seen in man for symptoms of heightened tonus of the musculature are often observed. The vermis is older phylogenetically and has quite different connections from the hemispheres of the cerebellum. Efferent pathways from the vermis end around the cells of the vestibular nuclei; efferent fibers from the hemispheres end around the cells of the red nucleus. Perhaps it is not too much to say that influences from the vermis inhibit tonus through connections with the vestibular nuclei while the hemispheres augment tonus through connections with the red nuclei. The cerebellar hemispheres are so closely connected with the cerebral cortex that their interrelations in respect to influence upon tonus will be discussed in another section.

5. *Control of tonus by the corpus striatum*

Pathological processes injuring the corpus striatum and subthalamic nuclei closely allied with it, produce marked changes in tone in man, for example, in paralysis agitans and the post-encephalitic Parkinsonian syndrome. The normal function of the corpus striatum in respect to the pattern of tonus is understood so poorly that it is useless to discuss it here. Later the abnormal findings in man will be summarized. Animal experiments have yielded no information concerning the physiology of the corpus striatum inasmuch as injury or total removal gives rise to no abnormal behavior.

6. The influence of efferent pathways from the cerebral cortex upon tonic reflexes

In man the activity of the central nervous system is dominated by the cerebral cortex. In connection with the development of this structure one well known efferent path has arisen, the cortico-spinal or pyramidal pathway which carries impulses directly to the motor cells of the final common path. This pathway has assumed great importance only in some Primates including man. Even in lower monkeys removal of the cerebral motor cortex produces no permanent paralysis.

With the development of Primates the cerebral cortex received a great impetus to further growth and differentiation. Elliot-Smith (1927) believes that the immediate impetus was the development of adequate stereoscopic vision. The positions of the eyes moved anteriorly and the snout decreased in size, permitting an overlap of the visual fields. This gave for the first time a picture of the world in three dimensions. At the same time the fore-legs were freed from their use as locomotor props and used to search for food and to carry it to the mouth and for climbing in the trees. With the rapid growth of the cerebral cortex the motor efferent pathway became further differentiated. Naturally it assumed particular control over the portions of the musculature that were being developed for skilled movements at that time. It influenced little the trunk musculature and the proximal portion of the limbs. A relatively large portion of the cerebral motor cortex is concerned with delicate movements of the hand. The paralysis following total injury of the cortico-spinal tract is, therefore, a selective one, involving the arm much more than the leg and the hand more than the upper arm.

Stimulation of the cortico-spinal pathway elicits short, quick contractions that are predominantly flexor in type. The execution of the delicate movements of the hands requires precise adjustments of tone in all the muscles around the joints. By what mechanism is this tonic adjustment made?

Two other cortico-efferent pathways, the fronto-pontine and temporo-pontine tracts, arise in the cerebral cortex. The fronto-pontine tract is made up of the axones of motor cells, somewhat smaller than the Betz cells, which lie anterior to the motor area particularly in the dorsal portion of the premotor area. The position of the cells giving rise to the temporo-pontine tract is not yet known. These tracts end around cells in the pontine nuclei and thus influences from the cerebral cortex reach the hemispheres of the cerebellum. It seems probable that these pathways are concerned in the finer adjustments of tone required to carry out the delicate movements initiated by the cortico-spinal pathway. In other words the cortico-spinal, fronto-pontine and temporo-pontine paths are initiated simultaneously for a precise coördination of finger movements.

If this is true it may seem strange that the functional significance of the pontine pathways in man was not clear from earlier studies. The reason for this is at once apparent. In the internal capsule the pontine fibers lie close to, and on either side of, the cortico-spinal tract. Any lesion here, and such a lesion is the most common cause of hemiplegia, would injure all these pathways together. Thus in hemiplegia, as will be demonstrated, there is a selective paralysis and hypertonicity of the muscles of the body. Clearly the only evidence of the different functions of these pathways could be obtained by injury to one without injury of the other. One possible position for such a lesion would be localized in the cerebral cortex involving the cells of one of these pathways but not of the others.

Adie and Critchley (1927) and many others have shown that a localized process in the dorsal portion of the frontal lobe, just anterior to the electrically responsive motor cortex, gave rise to characteristic abnormalities classified under three headings,—tonic perseveration, forced grasping and forced groping. In a number of cases small tumors are responsible for the changes and the damaged area was accurately localized at operation or post mortem examination.

The person with tonic perseveration finds that if he shakes hands or grasps an object with the affected hand he is quite unable to relax his grasp normally. Forced grasping is another form of this same condition. Sensory stimulation of reflex zones in the palm of the hand or on the flexor surface of the fingers will cause the fingers to close upon the stimulating object very tightly, nor can this vigorous grasp be relaxed. Forced groping is a more severe degree of this process. If the normal side of the body is examined the abnormal hand involuntarily tries to interfere in this procedure. Also when the patient finally succeeds in letting go of an object, the hand seeks for it and if possible picks it up again.

Obviously these are abnormalities in the regulation of tonus, extremely complicated in type inasmuch as the lesion affects the higher association areas of the brain. Animal experiments make these abnormal reactions more understandable. King (1927), Langworthy (A, 1928) and others have shown that, after removal of the premotor area in the cat, abnormalities in tonus of the striated muscles of the contralateral extremities are present. This suggests a control of the patterns of tone at the level of the cerebral cortex. This control becomes greater in Primates as the cerebral cortex becomes extremely differentiated and dominates the functions of the organism. As an objection to this theory concerning the rôle of the fronto-pontine pathway in the control of tonus, it may be said that no abnormalities in tone follow the extensive resections of the frontal lobe of man performed by the surgeons at the present day. The surgeon gives the motor cortex a wide berth and leaves the premotor area, of which we are speaking, intact.

Summary of the Control of Tonus by the Central Nervous System

Tone is fundamentally a stretch reflex, dependent on the proper functioning of the segmental apparatus of the cord or brain stem. Afferent impulses from the muscles influence the anterior horn cells and thus control tonus of the musculature.

All portions of the nervous system control this primary reflex arc, coördinating tone into patterns for balance, movement and locomotion of the animal. Afferent impulses from the deep neck musculature and from the vestibular and optic nerves are of particular importance, but as good a case could probably be made for other afferent stimuli. For example, following a loud sound the eyes and head are turned in its direction and the tonus of the whole body oriented in reference to the stimulus. The complicated ramifications of the sensory side of the reflex arcs cannot be followed, but it is possible to suggest the nervous system at which the reflexes are synthesized and the efferent pathways to the cells of the final common path. Thus the tonic neck reflexes are centred in the upper cervical segments of the cord and the vestibular at the level of the vestibular nuclei. Many afferent impulses influencing tone reach the red nucleus and the vestibular nucleus and these seem to be particularly important in regulating tonus. They not only produce patterns of tonus; the vestibular mechanism seems to augment the general tonus of muscles while the red nuclei have a control, predominantly inhibitory.

A further, more subtle control of tonus is effected through the suprasegmental portions of the nervous system. Injuries of the cerebellum in man produce hypotonia on the same side of the body as the lesion. The corpus striatum in man controls tone in some way that is not understood. Finally, the importance of the fronto-pontine and temporo-pontine pathways has been emphasized, acting through connections with the cerebellar hemispheres. Other cerebral efferent connections are undoubtedly also used in tonic coördination. Thus it has been suggested that optic righting reflexes are coördinated in the occipital lobes of the cerebral cortex.

It has been emphasized sufficiently that tonic control above the segmental level is always of the nature of patterns involving suitable adjustments for balance in new positions. Only at the level of the vestibular and red nuclei is there evidence of influences tending to augment or inhibit tonus as a whole. Succeeding portions of this review will discuss abnormal patterns of tonus in man released by injuries of the nervous system.

TREMOR IN RELATION TO ABNORMALITIES IN TONE

There is certain justification at this point in discussing tremors in man in relation to abnormalities in tone. The static and kinetic tremors of patients with cerebellar injuries, the intention tremor of multiple sclerosis and the tremor associated with abnormalities of the corpora striata and other basal ganglia may be considered.

Holmes (1917 and 1918) found that the tremors attributable to lesions of the cerebellum were always tremors of the unsupported parts of the body. If a patient is told to bring the forefinger of the affected hand within an inch of his nose and maintain it in that position a regular, almost rhythmical tremor of the whole arm quickly develops. Normally the arm would be maintained in this position by the tonic contractions of all the muscle groups. The tremor is due to absence of this tonus with the result that the new posture is maintained by voluntary contractions which fatigue easily. Fatigue causes the new posture to be momentarily relaxed; following the relaxation the posture is regained by voluntary contractions. This alternation of relaxation from fatigue and voluntary muscular efforts to maintain the posture is the causative factor of the static tremor.

Kinetic tremor is a prominent symptom of cerebellar defect especially when the lesion involves the brachia conjunctiva. Tremor may occur on any movement, the oscillations taking place in any of the planes of space. It is primarily the result of faulty fixation by antagonistic musculature.

The intention tremor of multiple sclerosis is similar to the kinetic tremor of cerebellar injury except that the oscillations in the latter condition are said to be less sharply defined. It is probable that they are due to identical defects since the tremor of multiple sclerosis is usually due to the involvement of cerebellar pathways, particularly of the superior peduncles and the mid-brain by the pathological process. The only result of injury to the red nucleus in man that has been observed is an intention tremor of the extremities of the opposite side of the body.

Walshe (1929) recently analyzed the tremor present following injuries to the corpus striatum and basal ganglia, for example, in paralysis agitans and the post-encephalitic Parkinsonian syndrome. The two leading abnormalities in these patients are hypertonus and tremor. The separation between the tremor and rigidity is not an absolute one for the cog-wheel phenomenon is undoubtedly to be attributed to a tremor which may not be clearly evident.

English clinicians have insisted for a considerable time that the tremor in these conditions is dependent upon the integrity of the cerebral motor cortex and cortico-spinal pathway. If a hemiplegia supervene in these cases the tremor disappears and never returns in the opposite extremities. Walshe then questioned whether the tremor was a new phenomenon produced by the lesion or whether it was evidence of normal activity of the nervous system unmasked by the injury. Many data favored the latter view.

The quick movements normally initiated by the cerebral motor cortex are not smooth contractions but are always carried out as a curve exhibiting waves. This may be observed by watching the slow flexion of one of the fingers and the irregularity may be recorded graphically. The rate of these waves is from eight to ten per second and they are observable only in slow movements. Moreover faradic stimulation of the cerebral cortex of animals at a rate of sixty per second produces similar waves of contraction in the musculature occurring at the rate of about ten per second. Denny-Brown (1929) found that the usual rhythm of clonus on cortical stimulation at rapid rates is from two to five per second.

Is there any relationship then between this normal cortical rhythm and the tremor present in paralysis agitans? If the tremor is followed in these cases and recorded graphically from its first appearance it is found to develop upon the basis of the normal waves of voluntary contraction and to be a simple accentuation of them.

Walshe suggests that the waves seen in the curves of slow voluntary contraction express a slow rate of cortical discharge while the disappearance of these waves in rapid movement is due to the more rapid rate of discharge from the cortex. In Parkinsonian rigidity without visible tremor this wave fusion occurs less readily and gives rise to the cog-wheel phenomenon while with manifest tremor the fusion does not occur at all or only when extensive and rapid muscular contractions occur.

In summary, the static and kinetic tremors of cerebellar disease and the intention tremor of multiple sclerosis are fundamentally attributable to abnormalities in tone. In like manner the tremor of paralysis agitans represents influences from the cerebral cortex unsupported by the normal tonic background.

ABNORMALITIES IN TONUS DEMONSTRABLE IN A PATIENT WITH HEMIPLEGIA

The next sections of this paper deal with common abnormalities of tone following injury of the central nervous system of man. The syndromes following partial and complete transection of the spinal cord have already been considered. The author obtained much of the material for the physiological analysis of hemiplegia and the Parkinsonian defects from a recent brilliant paper by Walshe (1929); he wishes to express his grateful acknowledgment.

Hemiplegia, one of the most common diseases seen in a neurological clinic, is also one of the most profitable conditions to interpret in terms of modern experimental physiology. All the abnormalities in the motor system and reflexes found in hemiplegia may be grouped under three headings, paralysis, abnormalities in tone, and release from cerebral control of reflexes presided over by the brain-stem and cord. The last group of abnormal reflexes need not concern us here except in so far as it is related to abnormalities in tone.

The paralysis following lesion of the pyramidal path has already been discussed from the standpoint of the development of the cerebral motor cortex. Since the impulses from this region do not exert an equal influence over the musculature the paralysis is a selective one. The facial muscles are the most severely paralyzed of any of the musculature innervated by the cranial nerves. Since the cells supplying the upper portion of the face receive bilateral pyramidal impulses, the upper portion of the face is spared. The extraocular muscles, muscles of mastication, muscles of the pharynx and larynx may show a weakness but no enduring paralysis after injury of one pyramidal tract. The injury is more severe in the case of hypoglossal innervation and the tongue usually deviates markedly toward the paralyzed side.

The paralysis is more marked in the upper extremity than in the lower and in both extremities is more severe in the distal than in the proximal muscle groups. In the arm, in order of severity, the paralysis affects all skilled and isolated movements of the hand and fingers, extension of the wrist and fingers, supination of the forearm, abduction and elevation of the upper arm. In the leg the weakest movements are dorsiflexion of the foot and toes and flexion at the proximal joints.

The amount of hypertonus does not necessarily correspond to the degree of spasticity. Cases of severe hypertonus are seen with a great deal of power of movement remaining, and the opposite often occurs. Under the heading of hypertonus, hyperactive tendon reflexes, clonus, and associative movements or abnormal patterns of tonus, may be considered.

The hypertonus, like the loss of voluntary movement, is not present equally in all the musculature of the body but is selective. In general, it may be said that the increased tonus is present in the flexor muscles of the arms and the extensor muscles of the leg. In the arm it is maximal in the flexors of wrist and fingers, the flexors and pronators of the forearm and the abductors of the upper arm. In the leg it is greatest in the plantar flexors of foot and toes, in the knee extensors and in the thigh abductors.

The quality of the spasticity may be learned by an attempt passively to flex the knee. The initial five or ten degrees of flexion can often be carried out quite without resistance. The muscle then develops tension and opposes the movement through another thirty degrees when this resistance melts away and the remainder of the movement is carried out quite easily. This is known as the "clasp knife" phenomenon.

The deep tendon reflexes are markedly accentuated in cases of hemiplegia. The knee jerk may be taken as an example. The quadriceps contracts strongly and there occurs a slow relaxation so that the graphic record shows a marked hump which is characteristic.

Clonus may sometimes be elicited from the hypertonic muscles. It is most commonly obtained from the gastrocnemius-soleus muscles as an ankle clonus and occasionally from the quadriceps as patellar clonus. It is rarely obtained from the musculature of the arm and when it is, may be produced by sudden stretch of the flexor muscles, particularly the wrist flexors. Clonus consists of alternate impulses of contraction and inhibition in the muscle. It may be stopped at once by relieving the stretch upon the muscle.

The associated reactions seen in hemiplegia are accentuations of patterns of tone controlled by centres in the brain-stem and cord. They are remarkably difficult to elicit in most cases but when present are of much physiological interest. They are usually demonstrated in the arm. During a yawn the hemiplegic arm slowly rises in front of the chest, the wrist extends and the fingers fan. This posture is maintained as long as the yawning lasts. Voluntary movements on the normal side are often associated with changes on the hemiplegic side. If the patient looks straight ahead and clenches the normal fist, the spastic arm which has been held adducted and slightly flexed at the elbow, wrist and fingers becomes further flexed. If the patient's head is first rotated toward the hemiplegic side, the spastic arm becomes extended and abducted. If the patient's head is rotated toward the sound side and he then makes a fist the spastic arm becomes increasingly adducted and flexed.

Acute rotation of the head against resistance toward the hemiplegic side sometimes produces extension and abduction of the affected arm, while rotation to the healthy side is followed by increased flexion and adduction. Similar changes in the position of the body may be produced by stimulation of the vestibular mechanism. Thus the hypertonus of hemiplegia is influenced by stimuli arising in the labyrinths, deep neck muscles and the arm muscles of the normal side.

These abnormalities of tonus present in the hemiplegic patient may then be analyzed in the light of our knowledge of the physiology of tone and its control by the nervous system. The hypertonus of the musculature is abolished by posterior root section or by intramuscular injection of novacaine in such strengths as paralyse conduction in the sensory nerves of the muscle and leave the motor pathway normal. It is dependent, therefore, on sensory impulses from the muscles themselves, and is a stretch reflex similar to that which has been discussed in connection with the segmental tonus mechanism of the cord. Moreover the hypertonus has many remarkable resemblances to that seen in the decerebrate animal.

The normal stimulus for the hypertonus is stretch of the muscle just as it is the stimulus for tonus in a normal muscle. The relative nonfatigability of the response depends on the asynchronous nature of the stimulus and upon the fact that the affected muscle fibers are the relatively slow contracting, fatigue resisting, red muscle fibers. If a muscle already engaged in a slight tonic contraction in response to a stretch be passively lengthened, its initial response is the development of increased tension, but as the lengthening is continued this response is inhibited, the tonic contraction ceases and the observer who has been manipulating the limb feels the resistance of the muscle melt and disappear. This explains the clasp-knife rigidity of hypertonic muscle.

If the two ends of the muscle are passively approximated and the muscle takes up a new and shorter length, it is again found to resume tonic contraction and to preserve the new posture passively imposed upon it. This is also a manifestation of the stretch reflex. For when the limb is released its weight tends slightly to stretch the shortened muscle and the stretch receptors are once more stimulated.

Walshe feels that the hypertonus found in hemiplegia is very similar to that present in the decerebrate animal. The distribution of tonus, however, is markedly different. In the animal the hypertonus is found in the extensor musculature or in the antigravity muscles. In the hemiplegic the increased tone involves the extensor musculature of the legs but the flexor muscles of the arms. The problem, therefore, is to explain the hypertonus of the flexor muscles of the arm in hemiplegia. Walshe points out that the arm in man is no longer used as a locomotor prop but hangs freely at the side. In this position it is the flexor muscles that are most stimulated by gravity and he believes that the tonic mechanism in the arm of man has been reset in the flexor rather than in the extensor muscles.

In confirmation he cites the study by Richter and Bartemeier (1926) of the decerebrate sloth. This animal hangs constantly by all four legs from the limbs of trees. In this position the flexor muscles are the antigravity muscles. After decerebration the hypertonus is present in the flexor musculature.

Since the removal of the cerebral motor cortex, even in the higher apes, produces no picture similar to hemiplegia or decerebrate rigidity in animals, it must be assumed that the cortico-efferent pathways in man have assumed a greater control not only over the contraction of muscles but also over the tone of muscles than in animals. This indeed seems to be the case. Even in laboratory mammals, however, injury of the fronto-pontine tract produces abnormalities in tonus in the contralateral leg musculature.

The question then arises as to whether hemiplegia is exactly equivalent to a half decerebration. Obviously the lesion does not remove the entire fore-brain as in experimental animals and it seems logical to speak of hemiplegia as a partial decerebration, or to say that hemiplegia produces physiological changes in tone similar to those seen in the decerebrate animal. Decerebrate rigidity was originally defined by Sherrington in terms of a level of the nervous system. "Decerebrate rigidity is a condition which ensues on removal of the fore-brain by transection of any of the various levels in the mesencephalon or in the thalamencephalon in its hinder part" (Sherrington, 1906).

Have any cases been reported of true decerebrate rigidity in man? In 1920 Wilson described tonic fits associated with marked extensor hypertonus of both the arms and legs. He suggested that a physiological decerebration was operative in these cases.

Walshe (1923) described a case of what he believed was a complete decerebration in man resulting from a supracellar cyst which pressed against the mid-brain producing the equivalent of an experimental transection. The abnormalities in this case were precisely those of a double hemiplegia. But the early pressure in such a case would first injure the cortico-efferent pathways in the cerebral peduncles and it is probable that this case was simply a quadriplegia and not a decerebrated man. It is probable that no true cases of human decerebration have been reported.

THEORETICAL AND PRACTICAL SUGGESTIONS FOR THE TREATMENT OF HEMIPLEGIA

The theoretical data that may be discussed in connection with the treatment of hemiplegia have already been developed in earlier portions of this paper and may be considered first.

The type of patient that would be selected for rehabilitation obviously would be a young individual who might be restored to a useful position in the community. The aged arteriosclerotics are thus excluded from consideration.

The most favorable prospects would be those cases in which a great deal of voluntary power remains in the muscles but in whom the hypertonus is so great that this power is inadequately utilized. Many cases of paraplegia due to luetic meningo-myelitis of the cord have a great deal of residual power in the legs which is overshadowed by the hypertonus. Even in capsular lesions the ratio between paralysis and spasticity is a variable one. Finally there are the cases of circumscribed cortical injury with abnormalities in tone but little or no paralysis.

Foerster (1913) believes that infantile paraplegias offer the best opportunity for improvement by therapeutic and operative procedures. In these cases there is often considerable latent voluntary power in the leg musculature, the extent of which cannot be gauged before the increased tonus is relieved. Unfortunately many of these patients show considerable corpus striatal and basal ganglia involvement. These are unfavorable for operative intervention for reasons which will become apparent in later portions of this review.

To return to theoretical treatment of uncomplicated hemiplegias or paraplegias, hypertonus is due primarily to the activity of the segmental mechanism, inasmuch as cutting the posterior roots abolishes the tonus. If Denny-Brown's theory is correct that neuro-tendonous endings in the muscle give rise to impulses that augment tone and the neuromuscular spindles to impulses that would inhibit tone, it would be logical to decrease selectively the activity of the neuro-tendonous endings. Unfortunately such a selective injury is at present impossible inasmuch as the tendon extends into the very substance of the muscle.

A review of the portions of the nervous system having a part in reflex tonus has suggested that tonus controlled by the cord as a whole or by the brain, consists in every case of patterns. However there is some evidence to suggest that certain portions of the nervous system tend to give rise to stimuli which augment tone as a whole. This is true of the vestibular nuclei. Moreover lesions of the cerebellum of man give rise to hypotonia. It seems quite possible that cutting one small tract in the brain stem might relieve the increased tonus entirely, leaving no abnormal tonic pattern. This is certainly worthy of the experimental test. Unfortunately the control of tonus varies so markedly in different animals that it would be difficult to apply the experimental results to man.

The more practical aspects of this problem may now be dealt with. Foerster has for years been interested in this problem and his results will form the basis of discussion. The reader is referred to a paper published in 1913 and to an address before the American college of Surgeons in 1930.

Since tonus is dependent upon the activity of the segmental reflex arc, Foerster proposed and carried out posterior root section in a number of cases. Obviously the question of the number of posterior roots that should be cut is a difficult one. One would wish to decrease the degree of tonus in the musculature to normal. Foerster found that in cases which showed an obvious hypotonia following the operation tone gradually increased and finally became greater than normal again. Moreover in cases where there was some voluntary movement, hypotonia of the extremity would not be desirable.

It is interesting to speculate concerning the effect upon voluntary activity of the legs if the pyramidal tract were still intact but all the posterior roots cut. Foerster maintains that in such a case the patient might learn to walk but as soon as voluntary attention upon the legs was relaxed for a second, tone would be lost in the legs and the subject would fall. A severe case of tabes with almost complete involvement of the lumbar and sacral roots would almost answer the description except that in this case the lesion is a gradually developing one.

Section of posterior roots for the relief of spasticity is, therefore, open to a number of objections. The tone may be decreased by the operation below the normal and may gradually increase until maked hypertonus is again present.

Posterior root section is particularly indicated in cases of paraplegia in flexion with no voluntary control of the musculature. Under these conditions the increased tonus may be so great that the muscles are extremely painful, and the pain is accentuated by cramp-like contraction of the musculature. Section of a number of posterior roots innervating the legs produces a hypotonia relieving the pain entirely. It also abolishes the annoying involuntary movements of the legs and makes it possible for them to be placed in an extended position which is much more satisfactory.

When certain voluntary power is present in an extremity posterior root section is certainly not the operation of choice. Here one should aim at correcting the deformity and decreasing the tone in the particular muscles involved. The results of this effort are usually more satisfactory in the case of the leg than the arm. Foerster is able to demonstrate in photographs and moving-picture films cases showing marked improvements.

The foot in hemiplegia is usually ventro-flexed and internally rotated. Lengthening the Achilles tendon and further tendon operations, if necessary, correct this deformity. The leg is greatly adducted and this adduction is so marked in cases of paraplegia that it, in itself, is a serious obstacle to locomotion. Section of the nerves to the adductor musculature of the thigh overcomes this contracture.

The problem of the hypertonicity in the quadriceps is a more difficult one. Foerster exposes the nerve to the muscle and cuts a few fibers at a time until the knee jerk is reduced to an approximation of normal. The knee jerk serves as a delicate test of the number of nerve fibers to be cut. Naturally the operation must be performed with local anesthesia.

By means of transplanting tendons and partially or totally deafferenting muscles good results may be obtained in the lower extremities when considerable voluntary power remains. Similar methods may be applied to the arm but there the technical difficulties are greater. Here, extension of the fingers may be obtained but the power of supination is difficult to restore.

It may be said that the position of the hypertonic extremities in a hemiplegic with great decrease of voluntary movement is perhaps ideal. The extended leg makes some degree of locomotion possible and the arm is held flexed out of harm's way. An extended and paralyzed arm would be much more of a liability than a flexed one.

Foerster believes that the hypertonicity develops in the muscles that are most relaxed, the flexors of the arms and the extensors of the leg. If Denny-Brown's hypothesis is again considered increased tone would develop in those muscles which are not stretched sufficiently to call the inhibitory impulses from the neuromuscular spindles into activity. Foerster found that the pattern of tone may be influenced in these cases by changing the position of the limb, particularly in patients in whom the excess tonicity was just developing. If the legs were kept flexed for a considerable period the spasticity was present in the flexor muscular. With our present physiological understanding of patterns of tonus we cannot attach too much value to this method of treatment. It is important, however, that from the first days of paralysis the fingers and wrists be held in extension by apparatus in order that they be as useful as possible. The same applies to the foot. In children, especially, a marked shortening of the achilles tendon should be avoided.

The operations suggested here require a great deal of time and skill. They are undoubtedly important in cases that are young and have a possibility of some voluntary function.

ABNORMALITIES IN TONUS DEMONSTRABLE IN PATIENTS WITH PARKINSON'S DISEASE

In the description of cases of paralysis agitans and the post-encephalitic Parkinsonian syndrome and in the physiological interpretation of the abnormalities, Walshe will again be quoted. Three marked symptoms are found in these patients,—tremor, rigidity and a decrease in the range and force of all movements. The tremor has already been considered.

The decrease in movement in these cases involves all groups of striated muscles although to various degrees. Walshe points out that there is a decrease in the force, rate and range of all voluntary movements. There is almost an extinction of movements of facial expression and of the limb gestures that accompany them and also a loss of all associated movements such as swinging the arms in walking.

It is further believed that it is the force required to carry out a movement that determines the degree to which the movement is decreased. Thus small amounts of force are required to produce the normal fleeting expressions of the face and these are diminished early, producing the mask-like face. Similarly associated movements require little force. The fact that facial expression and associated movements are not completely lost is clear from the fact that they appear again if the stimulus is great enough. Thus a smile may slowly spread over the face of the patient, remain an abnormally long time and then slowly fade again. Under stress of circumstances the patient may lengthen his stride and swing his arms in a free and rapid action.

Movements are slow in initiation and performance and are often brought to a stop before completion. In the performance of an alternating movement such as flexion and extension of the elbow there is a progressive decrease in speed and range of the movement until it degenerates to the oscillation of the tremor. In the case of a subject with paralysis agitans, an increasing effort is required to ensure completion of a movement.

The hypertonus found in these cases differs in several ways from that present in the hemiplegic. In Parkinson's disease the rigidity is not a selective one but involves all the musculature of the extremities and trunk, although it is more marked in the flexor muscles. It also is present in all the muscles innervated by the cranial nerves. It remains throughout the full range of movement and there is no decrease when the stretch on the muscle becomes great. The clasp-knife phenomenon, therefore, is not present. One does feel the tonus vary in a series of waves when the muscles are passively stretched. This is the cog-wheel phenomenon which is due to the presence of the underlying tremor. No stimuli from the neck muscles or vestibular mechanism cause the tone to change in any manner.

The two types of increased tonus do have features in common. In Parkinson's disease, just as in hemiplegia, the hypertonus is completely abolished in an extremity by cutting the posterior roots. Moreover injection of one per cent novocaine into the muscle in sufficient quantity to paralyze the afferent sensory mechanism and to leave the motor side of the reflex arc intact, abolishes the tonus. The rigidity in Parkinson's disease is, therefore, dependent upon a segmental reflex arc and the afferent stimuli from the muscles involved. Abnormal stretch of the muscle, on the other hand, does not elicit an inhibitory reflex. The deep tendon reflexes are active but seldom show the hyperactivity of hemiplegia.

The rigidity and decrease of voluntary movement have a close relationship to each other; indeed the damping down of voluntary movement is due to the difficulty of overcoming the rigidity of muscle. This was shown by Walshe very clearly. If the normal individual is asked to flex and extend the elbow an indefinite number of times the movement is continued for a considerable time before fatigue sets in and the range, rate and strength of movement remains relatively constant. In the case with rigidity the task is begun with a great deal of effort and the amplitude decreases almost at once so that after three or four beats the range has decreased to the extent of the oscillations of the tremor. Suppose now that novocaine is injected into the flexors and extensors of the elbow to paralyze the sensory endings and abolish the increased tone. The movement of flexion and extension of the elbow is now as free as in the normal individual. Hypertonus is here the factor that restricts voluntary movement and abolition of the rigidity makes free movement again possible. This explains why motor power is decreased in ratio to the normal force required to initiate it. When the stimulus is great enough movements break through.

The disturbances in gait are understandable in this same way. The rigid subject cannot adjust his centre of gravity with normal activity and his rapid short steps are an effort to preserve his balance.

THEORETICAL AND PRACTICAL CONSIDERATIONS IN THE TREATMENT OF THE PARKINSONIAN PATIENT

It has been found that certain drugs such as stramonium and hyoscin, when given to the patient in relatively large quantities, tend to decrease the rigidity of the musculature and, with the decrease in tone, voluntary movements become more free. Drugs have little effect upon the tremor. Cobb (1922) found that it was sometimes completely stopped by scopolamin.

There is, at the present time, no means of treatment for the tremor. It is known that section of the cortico-efferent pathways abolishes the tremor completely but this is scarcely useful in the way of therapy.

The fundamental abnormality in this condition is uniform hypertonus of the musculature and were this relieved, movements could be carried out quite easily and the patient would be normal except for the tremor. Perhaps investigations aiming to relieve the increased tonus might attempt surgical methods, cutting pathways which appear normally to augment generally the tonus of the entire musculature. Unfortunately animal experimentation is not an entirely satisfactory approach to this problem for reasons already discussed. However studies along this line should be carried farther.

SUMMARY

Injuries to the central nervous system of man often produce marked abnormalities of tone in the striated musculature of the body. The specific changes after lesions in certain definite portions such as the spinal cord or cerebellum have been discussed. Two common abnormalities, showing as prominent symptoms increased tonus, hemiplegia and Parkinson's disease were analyzed from the viewpoints of recent studies of the physiology of tone and possible treatment is suggested.

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THE SYPHILITIC OPTIC ATROPHIES

With Especial Reference to Primary Optic Atrophy

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Knowledge concerning the optic neuropathies due to syphilis is in a very confused state in the minds of ophthalmologists, neurologists and syphilologists alike. This confusion involves the clinical differentiation of the various forms of optic atrophy, the etiology and pathology of primary atrophy, the results of treatment, and even the propriety of treating at all. As in many other instances in medicine, the practical therapeutist has been in the forefront of the argument. Through his efforts, something of clarity is beginning to emerge as to the major problem of treatment results; stimulated by his partial successes and his failures, much study is being devoted, especially in Germany, to the equally important questions of etiology and pathology. It seems profitable, therefore, prior to a study of our own material of 200 or more cases of optic atrophy, to review the more important papers of the recent literature.

CLASSIFICATION OF THE OPTIC NEUROPATHIES AND THE IMPORTANCE OF SYPHILIS AS AN ETIOLOGIC FACTOR

Woods and Dunn (1923) provide a simple ophthalmologic classification of the optic neuropathies as follows: (1) primary atrophy, involving the entire nerve, without visible ophthalmoscopic evidence of any preceding inflammatory process; (2) secondary atrophy, including all cases with visible ophthalmoscopic evidence of preceding inflammation, i.e., obscuration of the physiologic cup, blurring of the lamina cribrosa, or deposition of connective tissue in the cup or on the nerve head; (3) retrobulbar neuritis, showing clinically diminished central vision with a normal fundus and nerve head on ophthalmoscopic examination, and having normal field outlines for form, or concentric contraction with the presence of central scotomas for either form or color; (4) atrophy especially localized in the papillo-macular bundle, with the clinical picture of pallor of the temporal section of the nerve, diminished central vision and normal field outlines for form but central scotomas for either form or color (this form of atrophy may often be regarded as the end result of retrobulbar neuritis); and (5) papillo-edema, including such entities as optic neuritis or neuro-retinitis clearly on an inflammatory basis, or choked disc due to increased intracranial pressure. Though this classification seems an easily workable one, it may be extremely difficult, even with the most careful ophthalmologic examination, to differentiate between the first four of these five groups.

Each of these optic neuropathies may be caused by a variety of etiologic factors, of which syphilis, though numerically the most important, is only one. In order to determine the cause, a painstaking general study of the patient must include, in addition to a detailed ophthalmologic survey, a complete physical and neurologic examination, special laryngologic study, antero-posterior and lateral stereoscopic roentgenograms of the head, blood Wassermann test, and examination of the cerebro-spinal fluid. In a series of 86 patients thus studied, gathered from the material of a large out-patient department of ophthalmology, Woods and Dunn found syphilis to be responsible for each of the types of optic neuropathy described, in the proportions shown in table 1, and to be the cause of the disturbance in 40 per cent of the patients studied. Twenty-five of the thirty-five instances of primary optic atrophy, or 71 per cent, were due to syphilis. Woods and Dunn illustrate the improvement in modern methods of diagnosis of syphilis by quoting an older similar study by Derby (1902), who found only 7.3 per cent of 108 cases of optic atrophy to be due to syphilis, while 67 per cent were of undetermined origin.

In a more recent report, Woods and Rowland (1931) have again reviewed the question of the etiology of the optic neuropathies. This second paper is based on a study of 137 patients hospitalized and studied in the wards of the Wilmer Ophthalmological Institute, and contrasts with the report of Woods and Dunn, which utilized a material of ambulatory patients. The incidence of syphilis as an etiologic factor is much lower in the second than in the first series, i.e., 16.7 as compared with 40 per cent. Increased intracranial pressure, due to brain tumor, pseudo-tumor, or intracranial aneurysm, however, increased in incidence from 11.6 to 34.3 per cent. The difference in percentage in these two groups is more apparent than real, and certainly depends on the fact that patients suspected of brain tumor are more seriously ill, and therefore better studied in the hospital than in the out-patient clinic. Even under these conditions, 20 of 48 patients with *primary* optic atrophy, or 41 per cent, were found to be suffering from central nervous system syphilis.

The incidence of syphilis in the causation of the various forms of optic atrophy may be roughly approximated by averaging the results of Woods and Dunn and of Woods and Rowland, as shown in table 2. It is at once apparent that primary atrophy is by far the most frequent type of optic neuropathy due to syphilis, and further that neurosyphilis is a more frequent cause of primary atrophy than all other etiologic factors combined.

TABLE 1

The etiologic classification of optic neuropathies (*Woods and Dunn*)

	TOTAL	CENTRAL NERVOUS SYSTEM SYPHILIS	SINUSITIS	BRAIN TUMOR	MULTIPLE SCLEROSIS	TOXIC AMBLYOPIA	SCATTERING	UNKNOWN
Primary atrophies	35	25	0	0	1	4	0	5
Papillomacular atrophies	24	4	5	1	4	5	1	3
Secondary atrophies	13	2	2	4	0	0	1	4
Optic neuritis	5	3	0	0	0	1	1	0
Choked disk	4	0	0	4	0	0	0	0
Retrobulbar neuritis	5	1	4	1	0	0	0	0
Totals	86	35	11	10	5	10	3	12
Per cent		40	12.7	11.6	5.8	11.6	3.5	13.8

A much less detailed but nevertheless important study showing the incidence of syphilis as a cause of optic atrophy is that of Harman (1922). Among about 4000 cases of blindness from all causes, he found in 65 blind infants 7 instances of optic atrophy, none of which were due to syphilis. However, optic atrophy accounted for 355 of 1855 instances of blindness among children of school age; and Harman says that no less than 230, or 65 per cent, of these optic atrophies, were due to congenital syphilis, most of which were associated with extensive disseminated choroiditis. It is a little difficult, in view of the careful studies by Woods and his collaborators, to believe the accuracy of Harman's statement that of 270 optic atrophies found in 925 blind adults, only 26, or 10 per cent, were due to syphilis.

The papers of Woods and his associates certainly represent the most careful recent study of the etiologic factors underlying the optic atrophies since the introduction of modern diagnostic measures; and the overwhelming importance they ascribe to syphilis as a cause of the lesion emphasizes the necessity for study of the syphilitic atrophies.

TABLE 2

The etiologic classification of the optic neuropathies (combining the series of Woods and Dunn and of Woods and Rowland)

	TOTAL CASES	CENTRAL NERVOUS SYSTEM SYPHILIS	ALL OTHER CAUSES	PERCENTAGE INCIDENCE OF NEUROSYPHILIS
Primary atrophies	83	45	38	54.2
Secondary atrophies	25	3	22	12.0
Retrobulbar neuritis	18	1	17	5.5
Papillomacular atrophies	47	5	42	10.6
Papilledema	50	4	46	8.0
Total	223	58	165	26.0

Igersheimer (1918) points out that syphilis may produce atrophy of the optic nerve in a variety of ways. (1) Atrophy may occur in association with disease of the bulb, especially the severe choroido-retinitis of congenital syphilis, or the severe intraocular inflammatory conditions of acquired syphilis. In such cases, it may be very difficult to decide whether the atrophic process in the nerve is secondary to, or merely simultaneous with, the intraocular condition. Jonas Friedenwald and the author have recently seen a tabetic with a severe choroido-retinitis and an apparent pigmentary degeneration of the retina associated with primary optic atrophy. (2) Atrophy may occur in gumma or periostitis of the orbit, due to direct pressure upon and resulting nutritional disturbances in the intraorbital portion of the optic nerve. (3) Atrophy may occur in patients presenting the neurologic picture of basilar syphilitic meningitis. Here the atrophy may be secondary, as the end result of papillitis, or apparently typically primary. If the latter, Igersheimer thinks (and a study of our own clinical material will subsequently afford confirmatory evidence) that it may be a matter of extraordinary difficulty to distinguish between primary atrophy due to tabes or to basilar meningitis. He points out that it is not uncommon for optic atrophy to be almost the only sign of neurosyphilis, and that positive findings in blood or spinal fluid do no more than to establish the diagnosis of neurosyphilis, not to differentiate its type. He believes that the diagnosis can often be made on the basis of collateral neurologic signs, or sometimes on the results of treatment, since he appears to feel that treatment is more hopeful in the atrophy due to basilar meningitis than in that due to tabes.[1] (4) Atrophy may be due to the pressure of syphilitic inflammatory products on the cerebral optic conduction apparatus anywhere from the orbit to the corpora geniculata externa. Igersheimer mentions as examples, pressure on the chiasm or the intracranial portion of the optic nerves by atheromatous vessels, gummas of the hypophysis, or hydrocephalus of the third ventricle. If there are atheromatous changes in the cerebral vessels on a syphilitic basis, the atrophy may be as much due to nutritional disturbances of the nerve as to pressure. Igersheimer cites a single case of Behr's, and mentions that Wilbrand and Saenger have seen similar instances, in which optic atrophy was apparently due to the pressure of syphilitic plaques in cerebral vessels on the nerve stalk. It is difficult to see, however, how one could differentiate clinically between atheroma of the vessels, or its effect on the optic nerves, due to syphilis or to the more frequent cerebral arteriosclerosis. (5) Finally one finds the genuine optic atrophy of tabes dorsalis, tabo-paresis and general paresis.

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[1] It is not uncommon to meet with the ophthalmologic criticism that if a given patient with optic atrophy improves, the atrophy could not have been primary. (Uhthoff, in discussion of Greef's paper, 1922.) This reminds one of the premalaria days of the treatment of paresis, when, if a paretic patient recovered under any form of treatment, someone could always be found to dispute the diagnosis of paresis. Once the idea has become fixed in the minds of physicians that a certain disease is incurable, it is almost impossible to overcome it unless by such overwhelming evidence as was provided by insulin, liver or malaria. Since no such evidence is as yet available for syphilitic primary optic atrophy, its supposed refractoriness to treatment is often wrongly included as a criterion of diagnosis. On the basis of our own experience, I do not believe it justifiable to assume that optic atrophy is due to basilar meningitis if the patient improves, or to tabes if he does not.

Since the primary optic atrophy associated with parenchymatous neurosyphilis (tabes and paresis) is by far the most frequent of these types and is also that about which there is most confusion, particular attention will be devoted to it.

THE FREQUENCY OF PRIMARY OPTIC ATROPHY IN NEUROSYPHILIS

Igersheimer's book provides a detailed review of the literature prior to 1918 and gives, on the basis of his own experience and that of others, numerous figures as to the frequency of optic atrophy in tabes. He estimates that from 10 to 15 per cent of all tabetics ultimately develop primary optic atrophy. He points out that while optic atrophy also occurs in paresis, it is much more frequent when the disease picture is of the tabo-paretic type than when the symptoms are purely cerebral. Wagner-Jauregg (1927) goes further than this and thinks that optic atrophy in paresis is very rare unless there are associated manifestations of tabes. In juvenile (congenital) tabes and paresis, Igersheimer says that optic atrophy occurs in about 50 per cent of the reported cases, though in many of these instances he thinks there may have been an associated cerebrospinal (mesoblastic) neurosyphilis as well as meta- (parenchymatous) syphilis.

The magnitude of the problem of primary optic atrophy is easily emphasized by the consideration of a few well-known statistics. The most reliable recent estimate of the incidence of syphilis in the general population of this country is that of Usilton (1930), based on actual surveys directed by the United States Public Health Service. She estimates that there are 423,000 new infections with syphilis each year. From these figures and the census reports of the numbers of the population of different age groups, it seems probable that at least 10 per cent of the adult population is infected. Of untreated or badly treated syphilitics, approximately 5 per cent develop tabes dorsalis (Mattauschek and Pilcz, 1913). Leaving out of consideration the question as to whether more modern treatment methods than those discussed by Mattauschek and Pilcz have done anything at all to decrease the incidence of neurosyphilis—and this is a subject which is as yet unsettled and on which there is violent debate pro and con—there must at any one time be more than half a million sufferers from tabes in the United States. If 10 to 15 per cent of these may be expected to develop optic atrophy, about 50,000 cases of this condition are constantly present. Since heretofore the expectation has been that all such patients progress inexorably to complete blindness, the economic importance of the question, to say nothing of its urgent and vital importance to the individual patient, is obvious.

Incidentally, it is worth noting that, according to John (1929), who has studied the admission diagnoses in a large ophthalmologic clinic, there has been no increase in syphilitic primary optic atrophy over the 20-year period 1905–1925. Whatever modern treatment methods of early syphilis may be doing to increase the incidence of other types of neurosyphilis, there is thus no evidence that it has had any effect on the incidence of optic atrophy.

It seems to be assumed by many ophthalmologists and neurologists, particularly of the older school, that if the patient has primary optic atrophy, the neurosyphilitic process must be tabetic in type. This assumption is no doubt due to the often made clinical observation, discussed by Igersheimer, that atrophy often appears as the earliest manifestations of tabes, in the so-called pre-ataxic stage. He cites the experience of Galezowski, who saw 55 cases of optic atrophy in the pre-ataxic stage of tabes, and only 8 in whom the atrophy developed after ataxia had appeared. Others, also quoted by Igersheimer, have even taken the extreme view that optic atrophy and the posterior column symptoms of tabes are mutually antagonistic, i.e., that the development of optic atrophy is more or less of a guarantee against the subsequent appearance of incapacitating posterior column symptoms and vice versa. However this may be, it is certainly true that one often sees primary optic atrophy as one of the earliest signs of tabes; that in such cases it may be many years before the appearance of the typical cord symptoms; and that in patients in whom severe cord symptoms are an early feature, optic atrophy is relatively unlikely to develop. If a tabetic with optic atrophy has or subsequently develops cord symptoms, the latter often progress very slowly, or come to an early and spontaneous standstill.

Igersheimer is willing to make the diagnosis of tabes on the basis of pupillary changes alone, especially anisocoria and the Argyll-Robertson phenomenon; and many other ophthalmologists and neurologists are apparently willing to diagnose tabetic optic atrophy in the complete absence of cord symptoms, with the idea in mind that such patients are in the pre-ataxic stage of tabes and will develop cord symptoms later. Others regard such patients as instances of optic atrophy due to basilar meningitis. On the basis of our own experience, we have become convinced that there is a large and important group of patients with primary optic atrophy due to neurosyphilis where the only neurologic abnormalities present are the atrophy and pupillary changes, that these patients cannot be diagnosed either as tabes or as basilar syphilitic meningitis, on clinical grounds or on the basis of laboratory evidence, and that the subsequent course of events does not aid in the diagnosis, because no other neurological lesions, and particularly no evidence of involvement of the posterior columns, may develop during many years of observation. This feeling is shared by Hawthorne (1922), who also raises the issue as to whether all cases of syphilitic primary optic atrophy are tabetic. He concludes, on clinical grounds only, that the process in the optic nerves is essentially the same whether or not evidence of posterior column involvement is, or ever becomes, apparent. This important question of the relationship of optic atrophy to types of neurosyphilitic involvement of the nervous system will be discussed subsequently, in connection with the pathologic anatomy of the optic neuropathies.

THE OPHTHALMOLOGIC DIAGNOSIS OF PRIMARY OPTIC ATROPHY

The best discussion of this question is provided by Igersheimer (1918). Primary optic atrophy due to syphilis is practically always bilateral, though one eye may be involved weeks, months, or even a year or two before the other. Unilateral involvement with the maintenance of normal visual acuity, field, and fundus on the other side over a period of years is extremely unusual, and Uhthoff (quoted by Igersheimer) saw only one such patient in a series of 300 tabetic atrophies. The ophthalmoscopic picture of atrophy is often discovered before the patient has had any symptoms of visual failure. The pallor of the disc usually involves the whole disc uniformly, though in early cases it may affect the temporal halves only. Secondary atrophy, with evidences of a pre-existing inflammatory process in the disc, is extremely rare; and likewise in primary atrophy there are rarely associated intraocular changes of co-existent or pre-existing inflammatory lesions. It is a curious fact that among the many patients who develop iritis, keratitis, choroiditis or choroido-retinitis, or even neuroretinitis as a part of early secondary or relapsing secondary syphilis, outspoken primary optic atrophy seldom develops subsequently. The retinal vessels show only slight alteration, if any, in the early stages; and later there is usually only a moderate amount of contraction, especially of the arteries.

It is a point of great importance, both in diagnosis and treatment, that the ophthalmoscopic picture of pallor of the optic discs does not always parallel the degree of visual impairment. This fact has impressed us more than once in our own material and has also been discussed by others. Traquair (1922) points out that it is often difficult to decide whether optic atrophy is or is not present, and that the diagnosis must sometimes rest on the skill and experience of the individual observer. He illustrates the lack of parallelism between pallor of the disc and visual impairment by citing the fact that in descending atrophies of the nerve from pressure or from disseminated sclerosis, visual loss often far exceeds the degree of pallor; while in early tabetic optic atrophy or in retrobulbar neuritis after recovery, the reverse may be true. Traquair thinks, quite correctly, that careful studies of the visual fields are indispensable in attempting to estimate the anatomic and physiologic damage. Dworjetz (1928) reports 9 patients who showed ophthalmoscopically apparent "total atrophy" of the discs but in whom visual acuity was absolutely normal. In practically all these patients, the visual fields for form were moderately to markedly concentrically constricted. The clinical picture from the ophthalmoscopic standpoint was primary atrophy in five, neuritic atrophy in three, and secondary atrophy after choked disc in one. The cause of the process is given as tabes in one, cerebrospinal syphilis in two, multiple sclerosis and sinusitis one each, and typhoid fever and methyl alcohol poisoning two each. Unfortunately, there are no data as to whether these patients subsequently developed any visual failure.

Dworjetz concludes that while cases with "porcelain white" discs without the slightest red color, but with completely normal vision, are rare, they do occur; and that it is not possible always to diagnose optic atrophy from the color of the discs, which does not always express the functional state of the nerve, nor is the color necessarily related to the number of degenerated nerve fibres present in an atrophic nerve.

In this connection, a recent paper by Somberg (1927) is of interest. This observer was associated with the Manhattan State Hospital where he states that he had the opportunity to examine the eyes of about 2000 paretic patients before and during treatment with tryparsamide. He found 86 patients who had pallor of the optic discs before treatment, the color ranging from pale orange to white. Seventy-three of the 86 had normal visual acuity (data as to visual fields not mentioned), while 13 had some loss of vision, but never less than 20/40, with practically normal fields. During a period of two years' observation, vision was reduced in 46 of these patients. In 22 it fell to 20/200 (or less?) with marked concentric constriction of the visual fields; and this visual failure often occurred suddenly, within a period of 2 to 3 weeks. In 24 others, vision diminished to a point between 20/50 and 20/100, without much alteration of the fields. The author regards this as evidence of progress in optic atrophy due to syphilis. Unfortunately his conclusion is based on uncritical reasoning, since he fails to state whether tryparsamide, a drug known to produce damage to the optic nerve (Woods and Moore, 1924), was used in these 86 cases; or whether similar visual loss, with or without the appearance of pallor of the discs, occurred in the remainder of the 2000 paretics, some of whom were and some were presumably not, treated with tryparsamide. The sudden occurrence of visual failure suggests that it may have been due to tryparsamide rather than to syphilis.

To continue with Igersheimer's description of the clinical aspects of primary optic atrophy, he points out that the associated changes in the visual fields are of the utmost importance for diagnosis because, in all probability, alterations appear in them before any change is apparent in the color of the discs. The first evidence of trouble is usually constriction of the fields for white and color, while normal central vision is retained. This constriction may be concentric, wholly irregular in outline, or it may take out one or another sector of the visual field. In the latter case the color fields show the same sector defects as the form fields. Frequently the constriction begins from above downward. There may be constriction of the color fields while the form fields remain normal. Igersheimer says that colors are progressively lost in the order: green, red, blue; and that when green perception is entirely lost the whole optic nerve is already involved. Enlargment of the blind spot is an early symptom. Scotomas, central or otherwise, are very rare. Igersheimer quotes Uhthoff as having seen them in 2 per cent of patients with tabetic primary optic atrophy, though always in association with peripheral field changes. Igersheimer himself and others also have, however, seen scotomas with normal peripheral fields in undoubted cases of tabes. Hemianopsias, binasal or bitemporal, also occur very rarely, though, as Igersheimer points out, these may be only a pseudo-hemianopsias due to symmetrical defects in the peripheral fields. As a matter of fact, the great rarity of hemianopsias in tabetic atrophies is, as will be subsequently mentioned again, a strong argument in favor of the fact that the primary pathologic lesion is in the optic nerve anterior to the chiasm.

Behr in numerous publications has laid great stress on disturbances of dark adaptation as an early, perhaps the earliest, diagnostic sign of tabetic primary optic atrophy. His opinions are strengthened by the confirmatory experience of Schindler (1922). The physiologic mechanism involved in dark adaptation, and practical methods for testing it, are reviewed in detail in a recent monograph by Adams (1930), and need not be described here. Much of the work on dark adaptation in syphilitic primary optic atrophy and in neurosyphilis generally, must be critically repeated, however, since Igersheimer (1928) among others has shown (1) that not all eyes with atrophic nerves show any disturbance of dark adaptation; and (2) that there is a fairly large group of tabetics with adaptation changes but no other evidence of damage to the optic nerve, that these changes apparently depend on the presence of disturbances in the pupillary light reaction, especially if the pupil is miotic, and that they disappear when the pupil is dilated by mydriatics.

From the clinical standpoint, the elucidation of many of the unsolved problems of optic atrophy requires a close working liaison between syphilologist and ophthalmologist. As will be subsequently shown, the success or failure of treatment depends very largely on the question of early and accurate diagnosis of optic atrophy. In this connection it is important to note that while ophthalmologists generally feel that visual field changes probably precede visual failure or pallor of the disc, nothing is known of the incidence of visual field defects in neurosyphilitics without loss of visual acuity and with normal discs. Certainly no detailed studies have been published since the introduction of modern instruments of precision for field taking. Igersheimer has examined routinely a large number of paretics and tabetics, and has found many (proportion not stated) with constriction of the visual fields. Unfortunately, he has been unable to follow such patients, and is unable to state whether such field defects always mean an incipient and later progressive atrophy, or whether, like the question of pallor of the discs, they may be merely "normal" variations without prognostic significance. Too little is known of the ultimate fate of neurosyphilitic patients with pale discs but without visual loss.

What is needed to clarify the clinical situation is the most careful study, from the standpoint of pathologic physiology, of the eyes of a large number of syphilitic patients in all stages of the infection and with all types of neurosyphilis, and such patients should be followed and the examination repeated at periodic intervals. Such a study would involve a great deal of time and the amassing of an enormous amount of negative information, but it would seem very much worth while. From the immediate practical standpoint, it seems urgently important that every neurosyphilitic individual, tabetic or otherwise, should have a detailed ophthalmologic examination as a part of the study of his case. Only in this way can optic atrophy be detected in its incipiency, and it cannot be too often emphasized, blindness can only be prevented by early treatment.

THE COURSE OF SYPHILITIC PRIMARY OPTIC ATROPHY

As already pointed out, the disease is almost always bilateral, though one eye may be involved some weeks or months, rarely a year or more, before the other. The patient may fail to note that he has practically monocular vision until his attention is drawn to it by accident. Usually, there is obvious pallor of the discs and a considerable alteration of the visual fields is already present by the time the patient first notices dimness of vision. In this country the reliance of the public on opticians and optometrists, rather than on ophthalmologists, often leads to the unfortunate result that by the time the patient reaches competent medical attention, he has already passed through a half dozen pairs of hands, has been fitted with glasses or had them changed, and vision is seriously injured. The course of untreated primary optic atrophy is progressively downhill, the average duration of the process from onset of symptoms to blindness being, according to Igersheimer, 2 to 3 years, with extremes of 2 to 3 months to as long as 12 years. As with other manifestations of tabes, there may be remissions of longer or shorter duration, though in our own experience this is unusual. The lack of uniformity in progress, and the possibility of spontaneous remissions, makes it extremely difficult to evaluate the effects of treatment, since in the individual case, one cannot be certain that the process would not have been equally as promptly "arrested," and remained quiescent for as long a time, as if nothing had been done.

THE PATHOLOGY OF OPTIC ATROPHY

Igersheimer (1928) summarizes the important questions to be answered by a study of the pathology of primary optic atrophy. These are: (1) In what part of the optic tract does the process begin? (2) In which portion of the optic nerve are changes first observed? (3) What relationship, if any, have inflammatory processes to the degeneration of the fibres of the nerve? (4) Are *T. pallida* actually present in the lesion, and if so, what importance have they in its production?

A clear understanding of the work which has been done on the optic atrophies presupposes some knowledge of the general pathology of neurosyphilis. There are various classifications of neurosyphilis on a clinico-pathologic basis, one of the simplest of which, as evolved by Stokes and Shaffer (1924) and by Moore (1927) from that of Head and Fearnsides (1914), is as follows:

1. Meningeal
2. Predominantly meningovascular (cerebrospinal syphilis)
3. Vascular
4. Parenchymatous
    a. Tabes dorsalis
    b. General paresis
    c. Taboparesis

It is obvious, from both clinical and pathologic standpoints, that it is often impossible to assign an individual patient with exactness to one or another of these groups. In almost all patients with clinical neurosyphilis of any type, there is evidence of some degree of meningeal, vascular, and parenchymatous involvement; and the most that one can say is that the type of involvement is predominantly meningeal or vascular, or meningo-vascular, or parenchymatous. In a general way, however, the classifications mentioned may be briefly described and their association with primary optic atrophy pointed out.

(1) Meningeal neurosyphilis—acute syphilitic meningitis, neurorecurrence—is usually an early manifestation of the disease, is characterized by an exudative and inflammatory process in the meninges, and involvement of the optic nerve is frequent in the form of optic neuritis or neuroretinitis. Necropsies on such patients are rare, since recovery is the rule. Reports are however available, from Pette (1924) for example. Treponemes have been found with relative ease and in considerable numbers in the diseased areas. Fuchs (1924) reports such a case, a patient who developed a neurorecurrence with bilateral neuroretinitis, and subsequently died of acute yellow atrophy of the liver after 19 injections of silver arsphenamine. At necropsy, there were no alternations in the optic nerve heads (probably healed by treatment), but in both nerves behind the eye there was a slight round cell infiltration and glial proliferation without meningeal changes. Treponemes were not searched for. It is particularly noteworthy, Behr states (1926), that in spite of the frequency of involvement of the optic nerves in such cases, this usually subsides (even if untreated) without the slighest visual disturbance or atrophy. This is contrary to current ophthalmologic opinion, which holds that such inflammatory changes in the optic nerves usually leave some evidence of atrophy, even though slight, in their wake. Behr claims to have shown that in syphilitic meningitis, degenerative changes in the optic nerves occur only when there is infiltration along the connective tissue septa and about the vessels within the nerve itself. He has seen instances in which the meningeal covering of the whole optic nerve was involved in a syphilitic inflammatory process but in which the interior of the nerve was penetrated only at one point. Degeneration of nerve

fibres was present at this point only, and not elsewhere. According to Behr, this is conclusive evidence that degenerative and atrophic changes of the nerve are not due to the influence of meningeal infiltration or thickening of tissues about the nerves.

There is also a late variety of syphilitic meningitis, in which the clinical symptoms are not so acute or fulminating as in the early variety. Pathologically, as described by Le Count and Dewey (1915), for example, this consists in a discrete or diffuse gummatous infiltration of the meninges, especially about the base. Clinically, the picture is diverse but is usually characterized by severe headaches, pupillary abnormalities, and often cranial nerve palsies. Some examples of apparent primary optic atrophy are associated with this type of neurosyphilis.

(2) The predominantly meningovascular or cerebro-spinal neurosyphilis is a catch basket classification from the clinical standpoint, including all patients who fail to fit clearly into one of the other groups. From the pathologic standpoint the lesions are diffuse, often multiple, and tend to involve more or less purely the mesoblastic elements of the nervous system, the meninges and the blood vessels. Since the discovery of modern staining methods for *T. pallida,* such as that of Jahnel (1924), far less attention has been focused on this type of neurosyphilis than on general paresis; but treponemes can often be found, especially in the perivascular infiltrations. It is questionable as to what extent primary optic atrophy is associated with this group.

(3) In a relatively small group of neurosyphilitics, the involvement is almost exclusively vascular, consisting of endarteritis and periarteritis, and is manifested clinically by hemiplegias, monoplegias, aphasias —cerebral vascular accidents often indistinguishable clinically from cerebral hemorrhage or thrombosis from other causes. Optic atrophy is an uncommon accompaniment of this type of lesion.

(4) The two great divisions of parenchymatous neurosyphilis, tabes and paresis, differ in many important respects from other types of neurosyphilis, not the least important of which is their relative refractoriness to treatment. Since the demonstration by Noguchi and Moore (1913) that treponemes could be found actually in the nerve tissues of the brain in paresis, a great deal of additional work has established the fact that not only are they present, but present in large

numbers (Jahnel). They may be found not only in the nerve tissues, but also in the meninges and about the blood vessels. Their presence leads to actual destruction of brain cells, though curiously enough, their localization is usually such that the intellectual functions of the brain, rather than its motor or sensory functions, are the ones to suffer. Cranial nerve paralyses are rare. As already pointed out, optic atrophy is uncommon in paresis unless tabes also exists.

In tabes, however, the situation is quite different. The primary lesion is probably not within the central nervous system at all, but in the posterior nerve roots. There is great debate, and a half dozen theories, as to the exact location of the primary lesion and the method by which it develops (Richter, Spielmeyer, Hassin, and others, summarized in a recent article by Stern (1929)). The spinal cord lesions and theories as to their origin need not detain us here. However, it is necessary to emphasize the point brought out by Stern, that in spite of many attempts to demonstrate *Treponema pallida* in the cord or root lesions of tabes, only four observers have been successful (Noguchi one case, Versé two cases, Jahnel one case, Richter 3 cases). In contrast to the rich spirochetal content of the brain in paresis, the nervous system thus seems singularly devoid of these organisms in tabes. Moreover, tabes is frequently associated with four isolated cerebral disturbances: first, and most frequent, the characteristic pupillary changes consisting of anisocoria, miosis, irregularity of pupillary outline, and disturbances of the direct or consensual reaction of the pupil to light; second, disturbances of sensation in the distribution of the fifth cranial nerve; third, oculomotor palsies; and fourth, primary optic atrophy. Only the first and last of these phenomena need concern us here. Ingvar (1928) has recently reviewed the question of the Argyll-Robertson phenomenon. He points out that the path of the pupillo-motor fibres is not definitely known, and that many hypotheses have been advanced to explain the lesion resulting in pupillary stiffness, the most famous of which is perhaps that of Bumke. This observer believes that the lesion causing loss of the pupillary light reaction is located in a small area between the terminal fibres of the branch of the optic nerves in the midbrain and the nuclei of the third nerve. Ingvar, as well as Hauptmann and others previously, point out, however, that the most painstaking search by many observers has never revealed any visible lesion in this area, and that treponemes have never been demonstrated in it. Even if this were not true, it would be difficult to explain the uniform selective invasion of this tiny area by organisms, especially since the neighboring nucleus of the oculomotor nerve, to say nothing of other cerebral areas, is so infrequently involved. Ingvar has evolved an explanation of the pupillary changes on the basis of a widespread rather than an intensely-localized lesion. He feels that in spite of the absence of definite evidence for the exact path of the pupillo-motor fibres in man, there are evolutionary and phylogenetic reasons for thinking that these pathways run on the surface of the diencephalon, and that many of the more superficial nerve fibres lack myelin sheaths. Exudative and inflammatory processes at the base are, he says, to some degree universal in metasyphilis (tabes and paresis); and the inflammatory lesions in the pia and arachnoid are in direct contact with the unmyelinated nerve fibres on the surface of the cerebrum (and also with cranial nerves). This contact leads to marginal degeneration of the optic pathways with resultant injury to the pupillo-motor paths. Ingvar thinks that this basilar meningitis may also be the explanation of the isolated ptosis occurring in tabes from injury to the fibres supplying the levator of the lid and the superior rectus muscle, which are known to run on the margin of the third nerve; and that it may also explain the early concentric constriction of the visual fields so often observed in optic atrophy, since unmyenlinated nerve fibres are also present in the periphery of the nerve. If necropsy shows no evidence of basilar meningitis in a metasyphilitic patient, Ingvar assumes that it was formerly present, and has left the various degenerative processes in its wake. The difficulty with this explanation is the fact that inflammatory processes at the base are not universal in tabes; indeed, so careful an observer as Richter thinks that they are rarely extensive and that if present in considerable degree, they indicate a co-existent paretic process.

Spielmeyer (1923) has shown that in metasyphilis, inflammatory and degenerative changes may exist side by side in the nervous system but that they need not necessarily be parallel in degree. While degenerative changes may occur as the result of inflammation they may also exist in high degree (as in tabes) without direct relation to it. To explain this phenomenon, certain investigators, notably Hauptmann

(1924, 1930), see a process at work in metasyphilis not dependent on the direct consequences of the presence of *T. pallida* in the nervous system. Hauptmann assumes that the nervous system is damaged by a toxin, dependent on the presence of organisms in the body outside the nervous system as well as within it. This toxin, whether it is elaborated by living treponemes, whether it arises from their extra-phagocytic destruction in the host's battle against them, or whether it is a substance normally present in the blood stream which is toxic for nervous tissues but under normal circumstances is prevented from reaching them, may gain access to the nervous system by an upsetting of the normal barrier between blood and spinal fluid, and this in turn may be due to the presence of few or many organisms within the nervous system. The apparent selective action of the hypothetical toxin for the pupillo-motor pathways and the optic nerve is no more difficult of explanation than the action of other and better known toxic substances, such as methyl alcohol or tryparsamide. It is impossible in a short space to give the detailed steps of Hauptmann's reasoning; one can only say that his theory explains many of the clinical and pathologic facts of metasyphilis which otherwise appear inexplicable with our present knowledge.

Stimulated by these and other considerations, Behr (1926) concludes that neurosyphilis may be still more simply classified into three main groups: (1) mesodermal neurosyphilis, involving brain or spinal cord (and including the meningeal, vascular, and meningo-vascular groups above); (2) ectodermal neurosyphilis—general paresis; and (3) metasyphilis—tabes dorsalis. He postulates that in the first two of these groups, the damage is done by the actual presence of *T. pallida* in mesodermal or ectodermal tissues; in the third, the lesions are dependent on the hypothetical toxic, as well as on specific, influences.

The modern study of the pathologic anatomy of primary optic atrophy begins with Stargardt (1913). Many earlier authors had held that the degenerative changes in the nerve were dependent on changes in the ganglion cells and nerve endings in the retina. Stargardt studied the visual apparatus of 19 patients with paresis, 4 with tabo-paresis, and 3 with tabes. Six of these (2 paretics, 3 tabo-paretics, and 1 tabetic) showed advanced optic atrophy; in four others, all paretics, there was outspoken partial atrophy. The findings did not differ materially in paretics and tabetics. The first and earliest evidence of damage to the visual pathways was in the chiasm, and consisted in an undue proliferation of glia fibres, with isolated plasma cells in the overlying pia. Next the intracranial portion of the optic nerves was similarly involved. With increasing infiltration of round and plasma cells in the covering pia, there was a simultaneous proliferation of glia fibres in the nerve. As time went on, the medullary sheaths underlying the infiltrated areas showed degeneration, and later the axis cylinders and fibrillae were also involved, to be finally replaced by glia cells filled with amyloid. The intraneural connective tissues and blood vessels showed no early alterations, and could be apparently normal even in far-advanced atrophy.

Stargardt states that in the 8 of his 26 patients who showed no degeneration in the optic nerves or chiasm, exudative changes in the covering pia were almost or wholly absent. However, as Wagner-Jauregg points out, exudative changes in the meninges of paretics, who composed the majority of Stargardt's material, are almost universal, and their presence or absence can be of little real importance for the development of optic atrophy.

Palich-Szanto (1917) studied 9 cases of optic atrophy, and agreed that exudative changes were present in the overlying membranes of the intracranial portion of the optic nerve, and the chiasm and that the degenerative changes were most marked in the periphery of the optic nerves. Here the nerve tissues were almost wholly replaced by glia fibres. The optic tracts and the geniculate bodies were wholly free from lesions. This observer made an extended search for treponemes, without success.

In Richter's (1912) three cases of optic atrophy in uncomplicated tabes, the findings were essentially the same as in Stargardt's patients. Richter agrees with Stargardt that the atrophic process in the nerves is the same in paresis as in tabes.

Fujiwara (1925) studied the visual pathways in 19 neurosyphilitics, of whom 10 had tabes, 2 probable tabes, 1 questionable tabes, 2 taboparesis, and 4 pupillary changes only. Clinical data as to visual acuity and the ophthalmoscopic picture were lacking in 5; in 12, vision and fundi were normal; and optic atrophy was present in two patients. From the pathologic standpoint, all patients except two showed some degree of atrophy of nerve fibres in the intracranial portion of the optic nerve, even including 9 patients with perfectly normal fundi during life. Some degree of atrophy was present in the chiasm in 11 cases; while in the tracts (investigated in 14 patients only), there were atrophic changes in 3 instances, including the two patients with optic atrophy; cellular infiltration of the overlying pia was found in all cases except two. The predominating cell was the lymphocyte; plasma cells were few. The degree of infiltration was variable but was regarded as moderate in all. Perivascular infiltration of the vessels running in the connective tissue septa of the nerve was present in 5 patients. Treponemes were not found in pia or nerve tissues in 11 cases studied. These findings are obviously contradictory of the earlier study of Stargardt.

Behr has been a deep, if very pessimistic, student of the question of primary optic atrophy. He examined (1926) serial sections, from the eye to the geniculate bodies, of 9 cases of optic atrophy. He concludes that the degeneration always begins at the nerve margins, in the nerve bundles underlying the pia. It may occur in a single area or in several simultaneously, and may progress at different rates. He believes that it always occurs distal to the chiasm, partly because of the great rarity of hemianopsia during life, partly because he has seen cases with moderately advanced atrophy of the optic nerves with only slight changes in and behind the chiasm. The intracranial portion of the optic nerve is, according to Behr, the portion of the visual pathways in most intimate contact with the pia and the mesodermal connective tissue septa. Exudative changes in these mesodermal tissues are constantly demonstrable in tabetic optic atrophy and absent in all other atrophic processes in the nerves. The primary change in syphilitic primary optic atrophy, Behr believes, is an inflammatory process in the connective tissue septa of the intracranial portion of the nerve and of the blood vessels which run within them. Such changes are demonstrable both early and late in the process. These changes, especially those of the blood vessels, impair the nutrition of the nerve fibres and degeneration results. Exudative processes in the meninges are, according to Behr, merely coincidental, as shown by their inconsistency. There are numerous instances of marked infiltration of the pia overlying chiasm and nerves, with no evidence of optic atrophy, and conversely, many instances of optic atrophy in the absence of such changes. Behr agrees with Richter that exudative meningeal changes at the base are rare in tabes, and that if present, they raise the question of a co-existing paretic process.

It seems to Behr important, and not elsewhere much stressed, that in early cases of optic atrophy the medullary sheaths are first affected, involvement of the axis cylinders coming later. He feels that this is the explanation for the fact that one can see the ophthalmoscopic picture of optic atrophy without any visual change except in the phenomenon of dark adaptation.

Behr believes with Hauptmann that a toxin of some sort must be partly responsible for optic atrophy, but does not agree that its effect is on the nerve fibres. He eliminates them partly because the apparent constant association of exudative changes in the mesodermal septa and vessels with degeneration of the nerve fibres is so unsystematic. Here and there throughout the central, intermediate, or subseptal portions of the nerve, one finds degenerated fibres surrounded by and in contact with others which are quite normal. If therefore the atrophic process is dependent on a toxin, some other portion of the nerve than the nerve fibres themselves must be susceptible to the toxin, namely, the glia fibres. In Behr's opinion the glia fibre system is the lymph supply system of the optic bundle and the nerve fibres are nourished through it. Morphologic and physico-chemical alterations in the glia fibre system result in a disturbance of nutrition in the nerve fibres and their medullary sheaths. If the nutritional disturbance is prolonged, death results, first of the myelinated sheaths, then of the axis cylinders. The primary change is, he thinks, not only in the marginal but also in the parenchymatous glia fibres; the secondary result is a disturbance of nutrition of the nerve fibres, ending in degeneration.

Igersheimer (1926, 1928) points out that on the basis of his own and earlier pathologic studies there is general agreement as to the facts that atrophy begins in the intracranial portion of the optic nerve distal to the chiasm, and that the degenerative process occurs first in the marginal fibres of the nerve. He states that there is as yet no agreement as to the third question of importance, i.e., the relationship of the degenerative to inflammatory processes. His own studies of the visual pathways of a large number of paretics, tabo-paretics and tabetics with and without optic atrophy, clarify this situation only in a negative sense. He found that round and plasma cell infiltration of the pia and arachnoid, near and around the chiasm and in the intracranial portion of the optic nerve, was frequent in both paresis and tabes, and this whether optic atrophy was present or absent. He regards it as important that in one case in which he could follow a beginning atrophy through the whole visual tract, there was only a very minimal cellular infiltration of the pia and none at all in the septa of the nerve.

Igersheimer has also provided the first reliable evidence as to the potential rôle of the actual presence of *T. pallida* in the causation of optic atrophy. Among 40 neurosyphilitics, the optic nerves were quite normal in 10 (7 paretics, 3 tabetics) and in one of these patients only, he found a single *T. pallidum* in the optic tracts within the brain. In 9 cases (8 paretics, 1 tabetic), there were marked inflammatory changes in the pia about the chiasm and nerves and in the septa, but no demonstrable degenerative lesions. Treponemes were demonstrated in the pia and arachnoid of the intracranial portion of the optic nerve in three of these patients (all paretics) and in one of the three also in the septa and glia. Optic atrophy was present in 21 patients (11 paretics, 4 tabo-paretics, 6 tabetics). Treponemes were demonstrated in 7 of these (once by dark field) in various parts of the visual apparatus, ranging from the meningeal coat of the intracranial portion of the optic nerve to the external geniculate body. In all, Igersheimer found the organisms in the visual apparatus (but never in the nerve tissues proper) of 1 of 10 tabetics, 2 of 4 tabo-paretics, and 7 of 33 paretics. To put it differently, treponemes were present in 3 of 9 patients with inflammatory but no degenerative changes, and in 7 of 21 patients with definite atrophy, or in exactly the same proportion whether optic atrophy was present or absent.

Pacheco e Silva and Candido da Silva (1929), in a paper which is accessible to me only in abstract, report that they studied the intracranial portion of the optic nerves of 21 paretics by Jahnel's technic. Unfortunately, the abstract does not state whether any of these patients had optic atrophy. In one case, many treponemes were found, within the nerve substance.

The net result of these elaborate pathologic studies by various competent observers has been to settle only the question of the point of origin of optic atrophy in the marginal fibres of the intracranial portion of the optic nerve distal to the chiasm. It seems obvious from what has been said that the atrophic process bears no direct relationship to the presence or absence of exudative changes in the overlying or neighboring meninges or to the presence or absence of *T. pallida*, in or close to the visual pathways.

After a consideration of these same data, Wagner-Jauregg criticizes the pathologic evidence by saying that a study of the visual apparatus in uncomplicated paresis (on which a good deal of the work has been done) is of no particular value for elucidating the problem of optic atrophy; even in tabo-paresis with optic atrophy the extensive pathologic changes in the whole brain render unsafe any conclusions as to such a highly specialized part of the brain as the visual tracts. What is needed is a study of pure tabes with optic atrophy, and most important of all, with beginning atrophy. This is obviously very difficult, since such cases rarely come to necropsy. He adds that any theory of the development of optic atrophy must fit this particular process to the pathologic and clinical entity of tabes dorsalis, and must explain such clinical facts as the frequent preataxic occurrence of optic atrophy, and its development in occasional cases of otherwise apparently stationary tabes, with negative serology of blood and spinal fluid.

The apparent lack of relationship between the presence of *T. pallida* in or around the optic nerves and the occurrence of optic atrophy has been one of the strong points of Hauptmann's toxin theory, and has also been advanced as a possible explanation for the relative inefficiency of anti-syphilitic treatment. Here again the evidence provided by the practical therapeutist is of some value. It will be presently shown in the section dealing with treatment that there are two forms of therapy which are sometimes successful in bringing about arrest or even slight improvement in the atrophic processes, namely, the subdural injection of various medicaments, and fever therapy. If optic atrophy is due to Hauptmann's hypothetical toxin, it seems reasonable to assume that the degenerative process will continue so long as the toxin continues to reach the optic nerves. Routine antisyphilitic treatment, judging by its failure to impede the downhill progress of optic atrophy, is not wholly successful in eliminating the toxin. It seems unlikely that the addition to routine treatment of such procedures as the intraspinal or intracisternal injection of neoarsphenamine, mercury, air, or arsphenaminized serum would cause a cessation of production of the toxin, which Hauptmann postulates is elaborated outside the nervous system rather than within it. On the other hand it is much more probable that the favorable result of the direct contact of medicated substances with diseased tissue means the destruction of living organisms responsible for the disease process. If this be true, it is necessary first to explain the presence of treponemes in optic nerves of neurosyphilitics without optic atrophy, and second, their apparent absence in many cases when optic atrophy exists. As to the former, it is of course well known that the tissue response to the presence of the *T. pallidum* in the nervous system is an exceedingly slow and chronic one, and that, for example, although the neuraxis is invaded by organisms within the first year of infection, the tissue response resulting in neurosyphilis develops so gradually that 2 to 25 years elapse before the appearance of symptoms. It is quite conceivable, therefore, that had the individual with treponemes present in a normal optic nerve lived longer, optic atrophy might have developed.

As to the latter, it has been frequently suggested that the spiral form of the *T. pallidum*, so well known to us, is only one phase in the life cycle of the organisms, and that the cycle may also include granular or even infravisible but nevertheless perfectly virulent forms. This hypothesis has recently received strongly suggestive support from the work of Levaditi, Sanchis-Bayarri and Schoen (1928), Warthin and Olsen (1930) and Saleeby and Greenbaum (1931). The first and last of these workers have described silver staining granules in testes and lymph nodes of rabbits and human beings, these tissues being known to be infectious for rabbits, but so far as could be determined by dark field and silver staining methods, free from the spiral form of the organism. Warthin and Olsen have observed in the aortas of syphilitics what they regard as a regular cyclical transformation of *T. pallida* under certain conditions to a minute granular form, all intermediate stages having been observed. They are as yet unwilling to accept the hypothesis that these granules represent the infective stage of the organism and a part of its reproductive cycle, but regard this as at least possible. Should further investigation establish the proof of this hypothesis, it seems probable that optic atrophy, as well as other lesions of neurosyphilis of obscure etiology, may be due to the direct invasion of the tissues by the variant form of the organism. This would also explain the beneficial effect of subdural therapy in optic atrophy and in certain other lesions of tabes.

One other aspect of treatment is perhaps of importance in the argument as to whether optic atrophy may be due to the actual presence of living organisms (in whatever stage of their reproductive cycle) in or around the optic nerves. This is the frequently repeated clinical observation that in some cases, treatment started with an arsphenamine product or with malaria results in prompt acceleration of the rate of visual failure. This has been emphasized by Behr, as one argument against all forms of treatment. From the clinician's standpoint, this would appear to be a typical Jarisch-Herxheimer reaction, entirely analogous to similar reactions in other tissues of the body, and thought to be dependent on the sudden destruction of organisms with liberation of their endotoxins.

THE EXPERIMENTAL PRODUCTION OF SYPHILITIC OPTIC ATROPHY

The only report dealing with this question familiar to me is that of Igersheimer (1918) who says he has seen degenerative processes in the optic nerves of rabbits "several times." He gives the protocols of 4 experiments in all of which the animals were infected by the injection of an emulsion of *T. pallida* into the common carotid artery. In three of the animals there were extensive lesions in choroid and retina, and the author regarded the accompanying optic atrophy as ascending in character, and due to the injury to ganglion cells. In one animal only was there a moderate degree of optic atrophy without marked intraocular changes; here the pathologic picture was quite similar to that seen in tabetic optic atrophy. The artificial conditions of these experiments makes them valueless for elucidation of the problem of optic atrophy in man.

Spielmeyer (1906) reports that in dogs infected with *Trypanosoma brucei*, optic atrophy developed in several instances, the apparently primary degeneration extending through the whole visual tract, from the optic nerve to the visual centers. In these cases there was no inflammatory change in the vessels. It is of extreme interest that in these dogs, there were also degenerative changes in the posterior columns of the cord and in the roots of the fifth cranial nerves, thus producing a close analogy to the lesions of tabes in man.

THE TREATMENT OF PRIMARY OPTIC ATROPHY

Only a brief perusal of the older literature suffices to show that the treatment of primary optic atrophy prior to the introduction of salvarsan was almost never of benefit, and that the process progressed inexorably to blindness at approximately the same rate as if nothing had been done. With the introduction of modern anti-syphilitic drugs, there began to appear individual case reports, some of which claimed improvement in or arrest of the process, while others spoke of the deleterious effect of the arsenicals in hastening the onset of blindness. Also, scattered here and there in communications dealing with the general subject of the treatment of neurosyphilis are allusions to partial successes or failures of treatment. I have made no attempt to collect material from all possible sources, preferring to limit this survey largely to papers which consider the treatment of optic atrophy as a special problem and which report a sufficient number of cases to lend weight to their conclusions.

Perhaps due to the prominence accorded the use of atoxyl as an antisyphilitic agent prior to the introduction of salvarsan, and subsequently in the treatment of trypanosomiasis, and to the fact that atoxyl was known to produce optic atrophy, the impression gained among ophthalmologists that arsenical drugs were potentially dangerous to the eye. This has been reflected in ophthalmologic literature in statements that the arsenicals should not be used in any form of ocular syphilis and should be especially avoided in optic atrophy. The feeling has not been lessened in the minds of ophthalmologists by the fact that no sooner had the agitation over atoxyl died down, than tryparsamide, an atoxyl derivative also having a deleterious effect upon the optic nerves in a certain proportion of cases, was introduced into the therapy of syphilis. Although many observers, important among them Igersheimer, have shown that arsphenamine (salvarsan) and its immediate derivatives have no deleterious effect of any kind upon any of the structures of the eye, and though this has been amply demonstrated in practice by the injection of millions of doses of these drugs, the belief persists, and is hard to eradicate. Recent articles by Zimmermann, Chambers, and Stokes have perhaps aided in clarifying this situation.

However, a few years experience with the arsphenamines in the treatment of optic atrophy sufficed to show that little more was to be gained from their routine use than by the older methods with mercury. Although one occasionally met with an individual success, usually in a patient with a very early atrophic process, it was never possible to be sure that this did not represent a spontaneous remission. In the vast majority of instances the speed of progress of the atrophy was not altered, unless for the worse, and blindness ensued as rapidly as if nothing had been done. Moreover, a considerable number of cases accumulated in which visual failure was promptly accelerated at the start of a course of treatment. Though these were usually patients with an already far advanced and rapidly advancing atrophy, the evident occasional production of a Jarisch-Herxheimer reaction in the diseased nerve and the sudden extinguishing of vision in such cases strengthened ophthalmologic opinion that the arsphenamines were contraindicated in optic atrophy.

Behr has been an indefatigable writer on the treatment of optic atrophy, and his opinions as to the results obtained from all standard methods of treatment except subdural or fever therapy, may be regarded as representative of ophthalmologic thought. He says (1926) that in more than 100 cases treated by him with mercury, bismuth and the arsphenamines, each alone, and in all possible combinations (except subdurally), and with the more modern fever therapy, including malaria, he has never seen a cure. There was never improvement in any genuinely progressive case, and all such patients became blind in the orthodox period of time. Although he has had no personal experience with the subdural method of treatment, he finds the results of others unconvincing. Not only has he failed to observe any beneficial result from treatment, but he has also seen visual failure accelerated by it, and he provides brief reports of 6 such cases treated by routine methods, and one additional case treated with malaria. As a result of these experiences, he thinks all treatment should be withheld from patients with primary optic atrophy, at least until the atrophic process has become complete.

John (1929) agrees with Behr. He has treated a large number of patients with optic atrophy, of whom he was able to follow only 28 for periods of time ranging from 2 months to 3 years. Twelve of these patients were treated with potassium iodide only; of these, six were "favorably influenced." The remaining 16 were treated with various other methods (no accurate details given); and of these only four were favorably influenced. Since no data are provided as to the degree of atrophy in the two groups, as to the type or duration of treatment, nor as to the rate of progress after treatment, one can only feel that John, like Behr, is unduly pessimistic in deciding from this experience that treatment is hopeless.

It is only fair to say that not only ophthalmologists, but many neurologists and syphilologists, including those of such wide experience as Stokes, for example, are therapeutic nihilists as to primary optic atrophy, having seen little benefit from treatment.

Behr points out with much justice that it is extremely difficult to evaluate the results of treatment in an individual case, since the rate of progress of the atrophic process is so variable (a few months to many years before blindness ensues), and since spontaneous remissions occur so often. As to the variability of progress, our personal experience is in thorough accord; spontaneous remission has, however, seemed to us to be a comparatively rare phenomenon. Behr also emphasizes the facts that to speak with any authority concerning treatment results, the investigator must have observed a large number of cases over a long period of time, so as to be sure that a favorable outcome occurs more frequently and lasts longer than in control untreated groups, and that many of the published reports so far have lacked essential ophthalmologic details. He insists that before, during, and after treatment the eyes must be under the careful observation of a competent ophthalmologist. The present study of the literature, though it leads to conclusions as to the efficacy of treatment diametrically opposite to those of Behr, support these contentions. The reasons for the prevailing feeling of hopelessness among ophthalmologists lie in the facts that it is difficult for any single observer to amass a sufficiently large series of treated patients followed for a long enough time; that reports appear as a part of the discussion of the general problem of treatment of neurosyphilis and are thus submerged in relative obscurity; and that few of them provide sufficient ophthalmologic details to be convincing to the reader. In dealing with a disease process the outcome of which has heretofore been uniformly unfavorable, it does not suffice for the investigator to report that he has treated so many cases with improvement or arrest. He must define his material sufficiently carefully so that the reader may be aware of the degree of atrophy and the approximate rate of its progress before treatment; he must not only describe his treatment method but provide details as to how much was given and over how long a period of time; and he must above all have followed his cases for years, not for weeks or months. Further reading of this paper will show to what extent the available published reports are deficient in these respects. However, it is unwise entirely to condemn a method of treatment as Behr condemns subdural therapy, because its users have failed to provide accurate ophthalmologic studies. Statements as to visual acuity are often available, and after all, the success or failure of a given method of treatment in any series of cases can readily be measured by ability to see versus blindness. If useful vision can be maintained for a period of years, it matters little to the patient whether there have been detailed records of visual fields, etc., throughout his illness.

In evaluating treatment results in optic atrophy, a certain amount of common sense is essential. It is quite obvious, from what has been said of the pathologic anatomy of the condition, that when a patient is completely blind and the fibres of the optic nerve completely degenerated, vision cannot be restored. Dead nerve fibres cannot be replaced by living ones. If our own experience is typical of that of others, it is a distressing fact that the majority of our own patients are blind or nearly so when first seen. If any form of treatment is to be successful, it must aim, not at the replacement of dead nerve fibres, but at the arrest of the degenerative process. The ultimate success or failure of treatment therefore depends entirely on whether it can be started while enough living nerve fibres remain so that, if they can be preserved, useful vision is retained. It is difficult if not impossible to set up a criterion of this applicable to every case. Certainly it cannot depend on the degree of pallor of the discs, since we have already seen that the discs may be chalky white in the presence of normal central vision; nor can it depend on the visual fields, for they may be concentrically contracted to a marked degree while central vision is normal or only slightly reduced. The standard which we adopted some years ago as a rough working basis (Moore 1927) is that if central visual acuity in the better eye is 20/60 or more, there is some hope of retaining useful vision, while if vision in the better eye is less than this, the prognosis is bad.

It is also important to determine whether there are other factors than the degree of visual acuity before treatment, which may aid in estimating the probable success or failure of a treatment method. The rate of progression of visual failure is certainly an important consideration to which little attention has been paid in the literature. It is unlikely that as good results can be obtained from any method of treatment in a rapidly progressive atrophy as in a patient with only slow visual loss. Many ophthalmologists have laid stress on the type of alteration in the visual fields as being an exact indication of the location and extent of the pathologic change in the optic nerves and as an indication for giving or withholding treatment. Wilbrand and Saenger (1913) differentiate two types of optic atrophy, one with gradual diminution of central vision and progressive contraction of white and color fields; the other with concentric constriction of form fields with normal central vision and maintenance of color vision in the functioning portion of the visual field. In the first of these, they believe the involvement of the optic nerve to be diffuse, and treatment to be contraindicated. In the second, the lesion involves peripherally placed fibres principally, and treatment is strongly advised. Behr (1916) describes three types of field changes in which he believes treatment to be contraindicated. These are (1) decrease in central vision and early loss of color vision with normal or nearly normal fields for form; (2) marked constriction of white and color fields with normal or nearly normal central vision; (3) slight changes only in form rather than color fields, normal or nearly normal central vision, but the ophthalmoscopic picture of advanced atrophy.

Arlt (1922), in describing the results of treatment with the arsphenamines, mercury, and potassium iodide, divides his 53 cases into three groups. Eighty-five per cent of his patients showed simultaneous bilateral loss of visual acuity, and constriction of form and color fields. This he interprets to mean diffuse involvement of the whole optic nerve. Here the prognosis was uniformly bad; treated or untreated, these patients progressed to complete blindness in an average period of about two years. A second group, comprising about 9 per cent of his patients, showed sector defects in the visual fields, instead of concentric constriction (no details as to visual acuity). In this group, the prognosis was relatively good. He separates into a third group those with central scotomas, who made up 6 per cent of his material. Here also the prognosis was fair for preservation of useful vision. Arlt brings out the point, more strongly stressed by Lederer (1928), that the outlook is much worse when vision fails in both eyes simultaneously than when treatment can be started while the atrophic process is still apparently unilateral, and visual acuity and fields in the better eye are still normal or nearly so. Unfortunately, Arlt gives no detailed information as to the type of treatment used, the number of cases treated or untreated, nor the results observed in the two groups. He did not employ subdural or fever therapy.

SUBDURAL (INTRASPINAL OR INTRACISTERNAL) TREATMENT

Prior to the introduction of the subdural method of treatment by Swift and Ellis (1913), the prognosis of optic atrophy was, as has been pointed out, completely or almost completely hopeless. Since 1915, however, there have been accumulating here and there reports which lend a different aspect to the situation. Swift and Ellis themselves reported arrest of the process in a few cases, using their technique of intraspinal arsphenaminized serum. Gennerich (1916, 1922), in Germany, also advocated intraspinal treatment with neoarsphenamine dissolved in the spinal fluid of the patient and reported several successful results.

In 1916, Schoenberg, in a long and carefully considered paper dealing with the anatomy and physiology of the central nervous system, carried out intravital staining experiments with trypan blue and lamp black. He found that these substances could not be brought into contact with the optic nerves or their sheaths when given by the intravenous route, but only when given by intraventricular (and cisternal?) injections. He considered the applicability of this fact to the treatment of optic atrophy, although he did not then report having used such a method of treatment himself. He accepted the idea that optic atrophy was due to the presence of *T. pallida* in the optic nerves, and felt that the only hope of relieving it was to introduce medicated substances subdurally. The ideas of Schoenberg (and many others preceding and following him) as to the relative impermeability of the nervous system and cerebrospinal fluid to drugs introduced intravenously have been abundantly confirmed. Schoenberg subsequently reported (1920) on the results of the intraspinal or intraventricular treatment of 8 patients with optic atrophy. In 3 the process was apparently arrested; five became worse in spite of treatment. Judged by present day standards, the amount of treatment given was inadequately small in all instances.

TABLE 3

Schacherl's results with intraspinal treatment in optic atrophy

CASE NUMBER	VISION BEFORE TREATMENT		VISION AFTER TREATMENT		PERIOD OF OBSERVATION	THERAPEUTIC RESULT
	O. D.	O. S.	O. D.	O. S.		
1	6/12	6/18	6/18	6/24	5 months	Slow failure
2	L. P.	6/18	Hand movements	6/24	4⅓ years	Arrest
3	6/6	Blind	6/5	L. P.	2⅔ years	Arrest
4	Cannot be determined from available data		6/38	6/8	10 months	Arrest
5	F. 1.5 meters	F. 2.5 meters	3/60	3/60	4⅙ years	Improved

In this and subsequent tables, L. P. = light perception; F. = fingers.

In 1923 Schacherl reported on the subdural treatment of primary optic atrophy in a series of 64 patients, of whom only 5 had been under observation for a long enough period of time to make him fairly certain of the permanency of his results. His treatment scheme was one of combined intravenous and intraspinal injections, the latter of 0.25 to 1 mgm. of neoarsphenamine dissolved in spinal fluid or salt solution. Such injections were continued (intervals not stated) until the spinal fluid serology had become negative and for several doses thereafter. His results were as shown in table 3. Of the remaining 59 patients in his series, 31 had been lost track of, 2 were completely blind when treatment was started, 22 were still under treatment, and in 4 treatment had recently been completed with good results though the observation period was too short to include them in the report. In a subsequent publication (1927), Schacherl confirms his earlier opinion, and states that his series now includes 31 cases "benefited" by intraspinal treatment. He gives no further details, and does not mention the number of unsuccessful treatment attempts. He states that there is no danger of aggravating the visual failure by this method of therapy, as a number of observers have suggested that there is with malaria, and he regards intraspinal treatment as the only method which holds out any hope for the patient with optic atrophy.

In 1924 Fordyce reports,

> "in optic atrophy the result of a basilar meningitis, the use of methods of treatment demonstrated to be futile or of little value is wasted effort and results in the loss of valuable time. Persistent intraspinal treatment when indicated by the fluid findings can arrest its progress and often preserve a useful amount of vision. Our statistics show 33 cases which were under observation and treatment, of whom 9 were of the simple optic atrophy type, 17 of the tabetic type, and 7 of the paretic type. . . . . Eight had almost complete atrophy of both eyes before treatment was undertaken. No amelioration was brought about. Seven had complete atrophy of one eye and partial in the other. Two of these progressed and 5 became stationary. Seventeen had incomplete atrophy of both eyes. In 12 improvement took place and the process was arrested. In 5 a further reduction of the visual field was noted before arrest of the process. The majority of these patients were controlled by well-known eye men, and the fields of vision taken before, during, and after treatment. In 5 of the patients arrest of the atrophy has been confirmed from two to five years after negative blood and fluid."

Stokes and Shaffer, on the contrary, reported in 1925 that, "In ten cases of the primary type (of optic atrophy) . . . . we were unable to record a single improvement. Occasionally, in the very earliest cases it seems possible to arrest the process." Unfortunately, it is impossible to tell to what extent, if at all, intraspinal treatment was used in these cases, though in a subsequent paper, Stokes says, "I have not . . . . seen intraspinal therapy . . . . produce results notably superior to those obtained by mercury and intravenous iodide."

Zimmermann in 1925 reported a careful study of 7 patients treated intraspinally. He draws a distinction between tabetic optic atrophy and that due to basilar meningitis, believing that the prognosis is better in the latter type. As already pointed out, however, it is probable that the process is essentially the same in either case, and that differential diagnosis is always difficult and often impossible. His results may be summed up as in table 4 (all patients with tabetic primary atrophy). It is worth noting that all of these patients were inadequately treated by present day standards. Data as to visual fields are provided in all: in general the fields were maintained at their original level, or nearly so, when vision was preserved.

TABLE 4

Zimmermann's results in tabetic primary optic atrophy

CASE NUMBER	VISION BEFORE TREATMENT		TREATMENT	VISION AFTER TREATMENT		PERIOD OF OBSERVATION	ZIMMERMANN'S SUMMARY
	O. D.	O. S.		O. D.	O. S.		
1	20/70	15/100	12 I.S.	20/30	Not given	3 years	Arrest
2	20/200	20/20	4 I.S.	10/200	20/20	2 years	Slow progress
3	20/25	20/30	12 I.V. 5 I.S.	20/20	20/20–3	Short duration?	Improved
4	20/30	20/50+	6 I.S.	20/60	20/60	2 years	Slow progress
5	20/40	20/70	16 I.V. 4 I.S.	20/200	20/100	5 years	Slow progress
6	20/25	20/25	6 I.S.	Blind	20/70	10 months	Worse
7	20/40	20/40	4 I.S.	H. M.	H. M.	2 months	Worse—due to treatment

Moore (1927) reported the results of intraspinal treatment in 20 patients with primary optic atrophy, in all but one of whom visual acuity in the better eye was as good as 20/60. He felt that if visual acuity in the better eye was less than 20/200 when treatment was begun, little could be hoped for from any form of therapy. In fourteen of twenty patients, visual failure was arrested. In five, visual acuity and fields were improved over the admission examination, and in nine, the process remained stationary. In a much larger series of cases treated with arsphenamine intravenously without intraspinal treatment or with mercury, bismuth and potassium iodide, every case progressed inexorably to blindness. These cases were reported as a part of a larger study of the results of treatment of central nervous system syphilis and details as to visual acuity, visual fields, amount of treatment given and the period of subsequent observation were not provided. A subsequent report of these and additional cases will supplement the present paper.

TABLE 5

The results of intraspinal treatment in optic atrophy (*Moore*)

CLINICAL RESULT	SEROLOGIC RESULT				
	Excellent	Good	Fair	Poor	Total
Improved	3			2	5
Stationary	4	1	2	2	9
Worse	1			5	6
Total	8	1	2	9	20

In 70 per cent a satisfactory clinical result was obtained.
In 40 per cent an excellent or good serologic result was obtained.
In 40 per cent an excellent or good combined result was obtained.

Gifford and Keegan (1927) have been utilizing the same principle of treatment, applied in a different way. They have employed intracisternal injections of 0.5 mgm. of bichloride of mercury (0.1 cc. of a 1/200 solution diluted with 15 to 20 cc. of the patient's spinal fluid). Certainly the cisternal route would appear to be preferable to the intraspinal, since it has been repeatedly shown that substances introduced into the cisterna magna reach the base of the brain sooner and in higher concentration than when they are introduced into the lumbar sac. This is probably true even when their diffusion from the lumbar area is aided by posture (elevating the foot of the bed). Gifford and Keegan report 14 cases of optic atrophy treated by this method. In one there was marked improvement, maintained for 5 years. In 5, the better eye remained unchanged and the authors consider the process to be arrested. Four patients were slightly worse and two much worse, but none were blind. The observation period ranged from 9 months to 5 years. One would like to know ophthalmologic details which are lacking.

Viner and McMurtry (1925) also report two cases treated by Gifford and Keegan's method. In one there was slight but definite improvement and in the other, the atrophic process appeared to have been arrested.

Meesman and Roggenbau (1929) treated 21 cases of optic atrophy (no details as to degree of visual failure before treatment) with a system including the intraspinal injection of 0.001 gm. neosalvarsan dissolved in spinal fluid, together with intravenous neosalvarsan and intramuscular bismuth injections. In two patients, the process was arrested; in one, vision failed rapidly during treatment. The remaining 18 all grew slowly worse, but the rate of progression of the atrophy appeared to be definitely slowed up. These patients were observed for periods of time ranging from 2 to 30 months.

Jaensch (1930) reports the results of subdural treatment with arsphenaminized serum (Swift-Ellis technique) combined with intravenous neoarsphenamine and intramuscular bismuth, in 18 patients. It is not possible to tell how much treatment he gave to any patient. He divides his patients into three groups: group 1, vision in the better eye more than 1/10, 7 patients, all blind in 2 to 27 months. No details are given as to how much better than 1/10 was visual acuity in any case. Group 2 included 7 patients in whom vision in the better eye was 1/10 to finger movements at 2 metres; of these 4 are stationary, or only slightly worse, and 3 are blind, in periods of time ranging from 4 to 24 months. Four patients were in group 3, in whom vision was less than finger movements at 2 metres, and two of these are slightly improved. On the basis of these results the author is pessimistic as to the outcome of treatment.

The same patients are discussed from the general standpoint in a separate communication by Rotter (1930), who omits all ophthalmologic details. Only 10 of the 18 were re-examined from the neurologic standpoint. Of these 5 are reported as stationary or improved, 4 slightly worse, and only 1 progressive. Presumably these results are an expression of the general neurologic, rather than the purely ophthalmologic, status. Rotter also reports in the same manner on the malaria treatment of 6 tabetics with optic atrophy. His findings are shown in table 6. The clinical and serologic outcome are only roughly parallel. Rotter is a strong advocate of the value of intraspinal or intracisternal treatment by the Swift-Ellis method in tabes, and especially in beginning optic atrophy, and draws from the same series of patients more encouraging conclusions than does his ophthalmologic confrère Jaensch. This is partly due, of course, to Rotter's more generally neurologic point of view.

TABLE 6

Rotter's results in tables with optic atrophy, expressed from the neurologist's standpoint

	NEUROLOGIC RESULTS*	SEROLOGIC RESULTS
Subdural treatment:		
Markedly improved	1	2
Slightly improved	2	14
Stationary	2	
Slightly worse	4	
Progressive	1	
Malaria:		
Markedly improved		1
Slightly improved	2	4
Stationary	3	
Progressive	1	

* Only 10 of the cases treated were re-examined.

Vranešić (1930) provides an interesting discussion of intraspinal versus intracisternal treatment. He takes the viewpoint that the cerebrospinal fluid represents a stagnant fluid system, and that foreign substances introduced into it diffuse upward or downward from the point of introduction by virtue of their specific gravity as compared with that of spinal fluid, and of the position of the patient. In rabbits, human cadavers, and living paretic patients, he has shown that if blood serum or a 10 per cent solution of sodium iodide is injected subdurally into the lumbar sac, the patient being kept sitting upright or in a horizontal reclining position, diffusion of serum proteins or of iodine into the cisternal or ventricular fluid takes place only very slowly and in small amounts. If, on the other hand, the injection is intraventricular or intracisternal, these substances appear promptly in the lumbar spinal fluid, and within a very few minutes are equal in concentration to that in ventricular or cisternal fluid. The explanation of this lies, of course, in the high specific gravity of blood serum (1027–1030) or 10 per cent sodium iodide (1082) as compared with that of spinal fluid (0.99–1.000). Utilizing this principle in treatment, Vranešić has further shown that when blood serum is injected intraspinally in the lumbar region, serum proteins can be made to appear promptly and in high concentration in cisternal or ventricular fluid provided the patient is placed in an appropriate position. Immediately after treatment, the patient is laid face down across a bed, his hips at the extreme edge of the bed, his weight being supported by his thighs and legs on the bed, and by his crossed forearms on the floor, his head cushioned by his arms. He is left in this position, almost literally standing on his head, for 15 to 25 minutes; then he is put to bed without a pillow, with the foot of the bed elevated about a foot, for 10 to 15 hours. Under these circumstances, it is interesting to note that the concentration of serum protein in the cisternal fluid rises from 0.3 per cent (Brandberg's method) to 3.7 per cent at the end of about 30 minutes and is maintained at this level for at least two and one-half hours.

Vranešić has treated 16 patients with optic atrophy by this modification of the Swift-Ellis technique. Of these, 10 were improved; i.e., in several, visual acuity before treatment improved by about one-third, others improved from 1/10 of normal vision before treatment to 3/4 afterward. Four patients remained stationary; and two others, both able only to count fingers before treatment, were uninfluenced and gradually became worse. No details were provided as to the amount of treatment given nor as to the length of observation. Vranešić is enthusiastic over the results obtained.

The whole question of the value of subdural therapy in neurosyphilis, whether intraspinal, intracisternal, or intraventricular, has been the subject of much debate, some of it acrimonious. The original unbounded enthusiasm was met by an equal complete scepticism of its worth. These opposing viewpoints led to a critical examination of the method by which this form of treatment affected its improvement, if any; and three main theories were elaborated. That the insignificant amount of drug introduced could be of any real treponemicidal importance seemed improbable, even if its treponemicidal activity were enhanced or supplemented by its presence in serum (Swift and Ellis). The subdural introduction of medicated serum, or of an irritating arsenical or mercurial drug, is known to set up a sterile meningitis; and it has been thought that this meningitis itself might be of therapeutic value, either from the hyperemia which it induces, or because its occurrence alters the permeability of the blood-spinal fluid barrier, so as to permit the entrance into the spinal fluid and nervous system of drugs introduced intravenously, and which otherwise would not reach the desired point in sufficient concentration to be of value.

The passage of time has also done much to clarify the usefulness of subdural therapy in various types of neurosyphilis. It is now more or less generally agreed that it is of value in certain selected cases, chiefly of tabes dorsalis, though there is as yet no agreement as to which of the three factors mentioned above brings about the improvement. Although in the present connection this point would seem to be of academic importance only, the question as to the mechanism involved has perhaps been reopened by the work of Horn and Kogerer (1929), and of Fazakas (1929). These observers, feeling that aseptic meningitis, whether because of hyperemia or because of disturbance of the blood-spinal fluid barrier, was the important feature of subdural treatment, undertook to produce a sterile meningitis by intraspinal or intracisternal air injections. Fazakas has treated 9 patients with optic atrophy by this method and Horn and Kogerer three. Inasmuch as the latter workers omit to supply such important details as visual acuity before treatment, and as they also used malaria almost simultaneously, their results are too unreliable to be quoted. Of Fazakas' 9 cases, adequate details are available in only 5. His method of treatment is to give an intracisternal air injection every 10 days, in the midst of a series of bi-weekly injections of neoarsphenamine (to a total of 5 to 7 gm.) or silver arsphenamine (to a total of 3 to 5 gm.). Recently he has been giving 0.5 to 3 mgm. of salvarsan intracisternally. All five of his patients had relatively early optic atrophy.

As a result of treatment, four were slightly improved and one was stationary after observation periods ranging from one to six years.

In table 7 an attempt has been made to summarize the results obtained from various modifications of the subdural method of treatment. The experience of certain investigators, such as Stokes, has been eliminated because of the absence of details. When sufficient details were available, I have also eliminated from consideration all patients who were blind before treatment was started, and a few more in whom various data were too inadequate to permit inclusion. The information provided does not permit any further analysis on the basis of visual acuity before treatment, visual field changes, or rate of visual failure. Generally speaking, classification as "improved" means little more than the less striking word "stationary," since in practically all patients the improvement, if any, was very slight, and consisted only in a slight increase in central visual acuity, or a slight widening of concentrically constricted form fields, or both. In 54 per cent of the 138 patients treated, a favorable outcome resulted, amounting essentially to the arrest of rapidly progressive visual failure, the progress being stopped over periods of time ranging from a few months to 5 to 6 years. It cannot be determined whether this arrest or improve-

TABLE 7

Summary of results obtained from subdural treatment

AUTHOR	TOTAL CASES	IMPROVED	STATIONARY	WORSE
Schacherl	5	1	3	1
Fordyce	24	12	5	7
Zimmermann	7	1	1	5
Moore	20	5	9	6
Gifford and Keegan	12	1	5	6
Viner and McMurtry	2	1	1	
Fazakas	5	4	1	
Jaensch	18	2	4	12
Schoenberg	8		3	5
Vranešić	16	10	4	2
Meesman and Roggenbau	21		2	19
Total	138	27	48	63
Per cent		54		46

ment left the patient with useful vision; obviously this was often not the case, since when treatment was started, the atrophic process was far advanced in many instances. Of the 63 patients classified as worse, some are blind, and in a few the onset of blindness was precipitated by treatment; but since blindness is certain if treatment is withheld, this cannot be urged as an objection to the treatment method. In general the rate of progress after treatment seemed slower than before.

Taking into consideration the fact that these figures include patients with all degrees of optic atrophy (excepting those already completely blind), these figures appear to provide incontrovertible evidence that something can be accomplished by the treatment of primary optic atrophy; and that in subdural therapy of one or another sort, a method is at hand which is well worth further study and much more extensive application. The data do not provide evidence as to the best method of treatment, whether intraspinal or intracisternal, whether arsphenaminized serum, neoarsphenamine or mercury dissolved in spinal fluid, air injections etc., nor can anything be said as yet as to the permanency of the results achieved, nor as to the type of patient most likely to be benefitted or harmed by treatment. It seems obvious, from *a priori* reasoning, that the earlier treatment can be started, the better the prospects of success; and that intracisternal treatment is likely to prove more effective than intraspinal. since its effect, of whatever nature, is produced in much greater intensity and in closer proximity to the diseased area. It is also apparent that one or a few treatments are not sufficient; on the contrary, it is probably desirable to continue at least until the serology of the spinal fluid is negative and perhaps for a few treatments thereafter. It must not be forgotten, as Rotter has emphasized, that the patient with optic atrophy has also other lesions of neurosyphilis, even though they may not be detectable clinically, and that he may have lesions of syphilis in important viscera outside the nervous system. Treatment for the average neurosyphilitic is measured in terms of years, not of a few months.

Much further study is needed and it is hoped that a survey of our own material, to appear subsequently, will be of service in answering some of the more obscure points as to treatment. In the meanwhile, enough has been said to show that subdural treatment of some type is of sufficient value to deserve much more widespread and patient trial, that nothing is gained by preliminary treatment with intravenous arsphenamine products, mercury, bismuth or the iodides, and that, indeed valuable time may be lost by failure to adopt subdural treatment immediately.

THE USE OF TRYPARSAMIDE IN OPTIC ATROPHY

Tryparsamide (n-phenyl glycine amide of p-arsonic acid) is a pentavalent arsenical drug which is of great value in the treatment of central nervous system syphilis. Like atoxyl, it has a tendency to produce visual damage in the normal eye, probably from elective localization in the optic nerve. About 10 per cent of all patients treated with it develop blurring or dimness of vision, and of these about half show objective evidence of visual damage in the form of concentric constriction of the visual fields and diminution of central visual acuity without scotomas. These changes precede any ophthalmoscopic evidence of atrophic changes in the nerve and if the administration of the drug is promptly stopped at their appearance, may spontaneously disappear in a few weeks or may persist for an indefinite time. In a few instances and even though the drug has been discontinued, there may be a very slow progression of field constriction and increasing pallor of the nerve, leading to confusion in diagnosis with a genuine primary atrophy due to syphilis. The only differential diagnostic points are that (1) visual acuity, fields, and fundi were known to be normal before treatment with tryparsamide; (2) the subjective complaint of blurred vision with accompanying constriction of the peripheral fields was known to occur suddenly and in direct relation to treatment with the drug; (3) the progress of the atrophy is very much slower than that of atrophy due to syphilis; and (4) central visual acuity usually returns promptly to its original level within a few weeks of the appearance of the reaction and remains normal in spite of slow progression of field constriction.

Woods and Moore (1924) observed untoward visual reactions from the use of tryparsamide in 21 per cent of patients with neurosyphilis and in only 6 per cent of patients in whom central nervous system disease could be excluded. Among neurosyphilitics, reactions occurred in 23 per cent of the tabetics and paretics treated (the type of neurosyphilis in which optic atrophy is most likely to occur), and in only 15 per cent of patients with other forms of neurosyphilis. These figures indicate that tryparsamide is more likely to produce visual damage in patients in whom the optic nerves are already actually (or potentially) diseased than in normal individuals. When the use of the drug was first begun, we felt (Moore, Robinson and Keidel, 1924) that any patient with pre-existing disease of the optic nerve or retina and especially with optic atrophy, should be rigidly excluded from tryparsamide treatment. Subsequent experience (Woods and Moore) showed that this was too sweeping a statement. Twelve patients with optic atrophy were treated with tryparsamide, and in only four was there any evidence of visual damage from its use. One of these four patients, an individual with slowly progressive optic atrophy of moderate degree, was precipitated into sudden blindness after the second dose of the drug. On the other hand, however, Woods and Moore saw nothing to indicate that tryparsamide was of marked value in optic atrophy. Though their patients were studied rather from the standpoint of the possible deleterious effect of tryparsamide than from that of the treatment of optic atrophy, they saw no improvement in any patient, and the progress of the atrophy did not seem to be arrested. Since that report, further experience has led us to take the position that, since other methods of treatment (subdural or fever therapy) are more likely to be productive of good results, and since tryparsamide is potentially dangerous even to the normal eye, its use in optic atrophy should be avoided unless other features of the individual case (the association of general paresis, for example) seem to render its use imperative, or unless the atrophy is already complete and the patient blind. If the drug must be used in a patient who still retains some vision it should be done only under the most painstaking ophthalmologic control.

Cady and Alvis (1926) administered tryparsamide to 16 patients with optic atrophy. Of these, three became blind, and in one instance this was apparently due to the drug; three others became definitely worse. In two cases, there seemed to be definite improvement, and in 8, the degree of visual impairment remained unchanged. No details are given as to ophthalmologic findings, or as to the length of observation. Although these results seem not dissimilar from those of Woods and Moore, Cady and Alvis arrive at opposite conclusions. While they recognize that patients with optic atrophy are more liable to injury by tryparsamide than those with normal eyes, they feel that the generally unfavorable results of other forms of treatment justify its use.

FEVER THERAPY IN OPTIC ATROPHY

The successful introduction of the malaria treatment of paresis by Wagner-Jauregg and his associates (1917) brought about, as with all new forms of treatment, its application to all other types of neurosyphilitic involvement. Many other methods of artificially producing fever have also been tried, as for example, relapsing fever, rat-bite fever, foreign protein injections, hot baths and diathermy. The favorable results of malaria treatment in paresis are well known; the results obtainable from the other methods mentioned are also good, but in general not quite as good as with malaria. In other types of neurosyphilis, however, and especially in tabes, the results of fever therapy have not been so striking as in paresis.

In optic atrophy, fever therapy has also been tried, and reports are now beginning to accumulate, especially from Germany. As mentioned above, Behr treated 6 patients with malaria. Five of these were already blind before treatment; in the sixth vision decreased within two or three days after the onset of malarial paroxysms from 6/7 to 3/60. On the basis of these six cases Behr, with his usual pessimism, condemns the use of malaria and falls back on what one can hardly fail to regard as his *idée fixe*, that all forms of treatment should be withheld.

Elschnig (1925) reports the results of malaria treatment in 18 patients with optic atrophy. In five, visual acuity before treatment ranged between 6/36 and 6/8 in the better eye; one of these patients became worse during the malaria, and in four, the process was arrested for 3, 3, 4, and 13 months, respectively. Six patients had vision ranging from finger movements to 3/36 in the better eye before treatment; all of these progressed. In 7, vision before treatment was limited to light perception; three of these became completely blind, and in 4 there was no change during a short observation period. Elschnig concludes that malaria therapy is advisable only in patients with early optic atrophy.

Fisher-Ascher (1926) reports on the status of these same patients (from Elschnig's clinic) at a slightly later date. There was no material change in the result in any patient.

Bering (1926) treated 6 patients with malaria. One early case was stationary about 2 years after treatment. Two others, more advanced, were also stationary about 18 months later. Three were worse, but in two of these the rate of downhill progress before treatment had been very rapid, while after treatment it was markedly slowed.

Wagner-Jauregg (1927) has treated a number of patients (exact number not given) and states that a small number improved slightly, and that in a larger proportion, the process was apparently arrested over a period of 2–3 years, though downhill progress before treatment was more or less rapid. In a still larger number of cases, the atrophy progressed after treatment, but Wagner-Jauregg thinks that the rate of its progress was considerably slower than before. He agrees with Behr that all other forms of treatment of optic atrophy (except fever therapy) are hopeless, but believes that the results obtained from malaria are sufficiently encouraging to warrant further extended trial.

Two cases are briefly mentioned by Fleischer (1927). One, treated with malaria, was apparently arrested two years later; the same favorable result, over a period of several months only, was produced in the second by relapsing fever.

Schacherl (1927) has also treated a number of cases with malaria and, although he provides no details, is unfavorable to the method because in some cases, rapid visual failure occurred during the course of the fever. He has seen such cases in which, after interruption of the malaria, visual acuity did not return to the pre-treatment level, or if so, only very slowly.

Sabbadini and Pisani (1928) treated 8 patients with malaria. The degree of atrophic change ranged from slight to severe. Within one to four weeks after completion of the malaria, reexamination showed definite improvement in four cases (especially in the visual fields), slight improvement in three, and one patient with an associated chorioretinitis was worse. They feel that in view of the hopelessness of other methods of treatment, malaria therapy should be tried in all cases, especially early ones. They recognize that the observation period in their cases was too short to permit definite conclusions.

Winkler (1928), in treating three patients with malaria, had an experience similar to the discouraging one of Behr, in that in one case, vision fell off so rapidly and alarmingly after the fourth paroxysm that the malaria had to be terminated. This experience, and the conflicting reports in the literature, lead Winkler to feel that another method of producing less intense fever, used in combination with anti-syphilitic drugs, might be worth trying. He therefore devised a method of administering 0.2 to 0.3 cc. of a 0.5 per cent suspension of sulphur in olive oil, at the same time giving an injection of bismuth. This procedure causes a rise in temperature to 100 to 101°F. He treated 5 patients with optic atrophy with this method (no further details given) and of these, 3 were stationary or slightly improved in 5, 8 and 18 months respectively.

This paper is mildly interesting in connection with a communication from Horn and Kauders (1928). These authors think that the cause of the sudden visual failure in patients with optic atrophy treated with malaria may be the very high temperatures. Inasmuch as they are of the school which holds that there is some other factor in malaria therapy than temperature responsible for the favorable effect in neurosyphilis, they feel that there might be less danger and more promise of a successful outcome in optic atrophy if malaria could be induced and carried through 8 to 12 paroxysms without such high temperature peaks. Accordingly they found that by giving 0.005 gm. quinine, on the two days following inoculation and the same dose daily after the paroxysms had begun, the temperature peaks could be held to 102°F. or less without interrupting the course of the malaria. The quinine is given during the period of defervescence. They treated 5 patients with optic atrophy by this method. There were no bad results and in 2 cases there was slight improvement. The post-treatment observation period was, however, very short.

Later reports than these are less encouraging. Havel (1929) treated 16 patients. He gives no details except that 9 became worse during or shortly after treatment, and in 7 there was no change after a short observation period. Wolff (1929) also treated 16 patients, and was unimpressed by the results obtained. Meesman and Roggenbau

(1929) observed a number of instances of optic atrophy in paretics treated with malaria. The results, so far as the optic atrophy was concerned, were uniformly bad. In two patients, vision failed rapidly during, and apparently as a result of, malaria. O'Leary and Brunsting (1930) have treated 15 patients, and saw no beneficial effects.[2] In all the progress of the atrophy was progressively downhill. In the 6 cases reported by Jaensch (1930), the results were uniformly bad.

Hessberg (1930) reports on 11 patients treated with malaria. His results are capable of summarizing in tabular form. One patient was improved, two were stationary and 8 were worse; but the speed of progress of the atrophy seemed to be slowed up.

TABLE 8

Hessberg's results with the malaria treatment of optic atrophy

CASE NUMBER	VISION BEFORE TREATMENT		VISION AFTER TREATMENT		PERIOD OF OBSERVATION	SUMMARY
	R.	L.	R.	L.		
1	H. M.	5/5	H. M.	5/5	3 years	Stationary
2	5/4	5/4	2/35	5/20	5½ years	Worse
3	5/4	5/4	3/50	5/25	2½ years	Worse
4	5/10	5/25	5/5	5/20	1 year	Better
5	4/50	5/50	1/25	4/36	4 months	Worse
6	H. M.	H. M.	Sl. impr.	Sl. impr.	1 month	
7	8/50	H. M.	H. M.	H. M.	6 months	Worse
8	5/5	5/5	4/10	4/7	1⅓ years	Worse
9	5/5	1/100	5/5	No data	1⅓ years	Stationary
10	L. P.	5/35	L. P.	L. P.	4 years	Worse
11	5/8	5/12	5/15	5/20	4 years	Sl. worse

One or two investigators, among them Wurz (1927), Lederer (1928), and Hessberg (1930), have attempted the treatment of optic atrophy with fever producing injections of foreign protein with or without the administration of anti-syphilitic drugs. Wurz reports 7 cases treated with typhoid vaccine intravenously. He saw no deleterious effects from treatment. One patient was blind before treatment, four could see only finger movements, and two had as much as 6/50 vision in the better eye. Obviously, all had far advanced atrophy. Of the 6 with some vision before treatment, one was slightly improved, four were unchanged, and one worse after treatment. The period of observation is not stated.

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[2] In a personal communication (November 25, 1931) O'Leary is less pessimistic, as follows: "Since we started the use of malaria in 1924, we have treated 26 patients with tabes dorsalis complicated by optic atrophy. In none of these cases was the vision improved, although in 9 there has been no appreciable decrease in vision since the fever treatment. In 8, what vision the patients had at the time of treatment has disappeared and they are practically blind at this time. I have insufficient data on the rest, but none of the group showed any improvement whatsoever. One striking feature is the fact that all of the patients in whom there has been an apparent arrest of the optic atrophy were in the early stages of optic atrophy when the malaria treatment was given."

Lederer reports the results of phlogetan treatment in five patients, summarized in table 9. Phlogetan is a proprietary German remedy, protein in nature. The author emphasizes the fact that in his cases 1, 2, and 3, the optic atrophy was to all intents and purposes unilateral, vision in the better eye being normal or nearly so before treatment. He points out that in these three cases, the good result obtained has persisted 3, 4 and 5 years respectively. He feels that since unilateral optic atrophy, the better eye remaining uninvolved, is so extremely rare in an untreated material (Uhthoff saw only 2 such instances among 300 patients), the preservation of vision in the better eye in his three patients can only be interpreted as a result of treatment. This being so, such patients offer the most favorable prognosis.

TABLE 9

Lederer's results in optic atrophy from non-specific fever therapy

CASE NUMBER	VISION BEFORE TREATMENT		TREATMENT	VISION AFTER TREATMENT		DURATION OF OBSERVATION	AUTHOR'S SUMMARY
	O. D.	O. S.		O. D.	O. S.		
1	Blind	0.7	Sodium nucleinate, arsphenamine, phlogetan	L. P.	0.7	4 years	Slight improvement
2	L. P.	0.9	Phlogetan Neosalvarsan	Blind	1.0	3 years	Arrest
3	H. M.	0.7	Phlogetan Neosalvarsan	Blind	0.9	5 years	Arrest
4	Blind	0.6	Phlogetan	Blind	0.5	9 months	Slightly worse
5	0.2	0.3	Phlogetan Malaria	F. M.	F. M.	3½ years	Worse

Hessberg (1930) has used non-specific fever therapy (with pyrifer, another German proprietory product) in patients whose general physical condition precludes malaria. He reports 5 cases, one of whom, with normal central visual acuity before treatment, has remained stationary for 5 years. Two others, with vision in the better eye 5/5 and 5/35, respectively, were unchanged one month after treatment. Two others with vision in the better eye 5/15 and hand movements respectively, became blind during treatment.

The results of various observers with malaria in optic atrophy are summarized in table 10, patients blind before treatment being eliminated. As in the case of subdural therapy, the work of several investigators is omitted from the table because of incomplete data. Of the 89 cases included, a favorable outcome was obtained in 35, or 39 per cent, while 61 per cent progressed in spite of treatment. These results are not as good as those obtained from subdural treatment (54 per cent favorable outcome); and in addition, two factors important to a comparison of the two methods of treatment cannot be presented in tabular form. In general, the patients who have done well after malaria have been observed for a much shorter subsequent period of time than the similar group after subdural treatment. In addition, there is general agreement that malaria is much more likely

TABLE 10

Summary of results obtained from fever therapy

AUTHOR	TOTAL CASES	IMPROVED	STATIONARY	WORSE
Behr	1			1
Bering	6		3	3
Winkler	1			1
Horn and Kauders	5	2	3	
Hessberg	11	1	2	8
Jaensch	6			6
Sabbadini and Pisani	8	7		1
Elschnig	18		8	10
Fleischer	2		2	
Havel	16		7	9
O'Leary and Brunsting	15			15
Total	89	10	25	54
Per cent		39		61

to precipitate sudden visual failure during its course than is the subdural method of treatment.

General considerations affecting the choice of a treatment method in optic atrophy. The results just described from subdural or from fever therapy are an adequate answer to the question as to whether patients with optic atrophy should be treated, or permitted to progress to blindness untreated. Obviously, when the progress of visual failure can be arrested in 40 to 55 per cent of the cases, the responsibility of the physician who withholds treatment is a heavy one. The inadequate ophthalmologic data provided by most of the investigators in this field make it impossible to draw accurate conclusions as to the probable success or failure of any treatment method in a given case, except on the basis of the degree of visual failure present. If one analyses the reports of Schacherl, Zimmermann, Lederer, and Hessberg, one arrives at the data shown in table 11. Here is confirmed the *a priori* conclusion that the earlier treatment can be started, the better the result. Of 17 patients in whom vision in the better eye was 20/40 or more, the process was arrested in 10, or 58 per cent; while among 11 patients in whom vision in the better eye was less than 20/40, good results were obtained in only 4, or 36 per cent. These figures combine the results of subdural and fever therapy.

TABLE 11

The material of various investigators shows better results in early than in advanced optic atrophy

VISION IN THE BETTER EYE BEFORE TREATMENT	TOTAL CASES	AVERAGE PERIOD OF OBSERVATION	RESULTS OF TREATMENT	
			Improved or stationary	Worse
20/40–20/20	17	3 years	10	7
Less than 20/40 but not worse than finger movements	11	2⅓ years	4	7

Enough has been said to make it quite clear that it is useless to expect any result from routine anti-syphilitic treatment. Bismuth, mercury, or the iodides will not impede the progress of nerve degeneration in the slightest. It is unwise to employ them as Stokes suggests, as preparation for subsequent arsphenamine, in the effort to avoid a Jarisch-Herxheimer reaction. By doing so, valuable time may be lost, and further visual failure which cannot be remedied may occur. The various subdural methods of treatment described all involve the use of intravenous arsphenamine, and in a small number of cases, the first injection of this drug may produce a Herxheimer reaction and rapid visual failure. This usually occurs, as already pointed out, in patients with far advanced and rapidly progressing atrophy. In any case, it is a risk worth accepting, since without treatment blindness is certain in any event.

From the purely ophthalmologic standpoint, one may summarize the situation as follows: subdural treatment, probably best given by the intracisternal route, offers something better than an even chance of arrest of the process provided vision in the better eye is 20/40 or more. If it is less than this, the chance of preservation of useful vision is considerably less; but the attempt is still worthwhile as long as any vision better than light perception persists. There is only a small risk that this form of treatment will accelerate the visual failure. Malaria, or other forms of fever therapy, had better be reserved as a last resort, to be used in patients whose vision has continued to fail under subdural treatment, or who, for one reason or another, cannot be given arsphenamine products. Malaria is somewhat less likely to bring about arrest of the process than subdural treatment; sufficient time has not yet elapsed to make it sure that the arrest produced by it is as lasting as that following subdural treatment; it is much more likely suddenly to accelerate the rate of visual failure than the subdural method; and it carries with it a distinct hazard to the life of the patient.

It must be borne in mind, however, that the choice of a treatment method often depends, not on the ophthalmologic status, but on the evidences of syphilitic involvement of other portions of the nervous system than the optic nerves, or of other parts of the body than the nervous system, or on the presence of complicating non-syphilitic diseases. Each patient represents an individual problem, to be solved by the combined efforts of the ophthalmologist and the syphilologist.

SUMMARY

The unsettled problems of the syphilitic optic atrophies involve the clinical differentiation of their various forms, their incidence, early diagnosis, mechanism of development, pathology, and treatment. About 90 per cent of the syphilitic optic atrophies are of the primary type; and syphilis outnumbers all other causes combined in the etiology of primary optic atrophy. Apparently primary optic atrophy may occur in association with various types of neurosyphilis, most commonly with tabes dorsalis. It may be due to the pressure of syphilitic inflammatory tissue upon and resulting nutritional disturbance in, the optic conduction pathways, as from orbital gumma or periostitis, or from basilar meningitis. It may occur as an almost isolated phenomenon in neurosyphilis, without associated clinical evidence either of tabes or of meningitis.

It is estimated that about 50,000 patients with syphilitic primary optic atrophy are constantly present in the United States.

The diagnosis of primary optic atrophy cannot be made with certainty on the basis of pallor of the optic discs, which may not parallel the degree of visual improvement. Alterations in the visual fields, and in the phenomenon of dark adaptation, both of which probably precede changes in the color of the disc, are more important elements in diagnosis. Nothing is as yet known as to the incidence of these abnormalities in an unselected series of patients with neurosyphilis, nor of their prognostic importance when their appearance precedes subjective symptoms of visual failure. Since hope in the treatment of optic atrophy depends on early diagnosis, however, close cooperation between syphilologist and ophthalmologist, and the routine ophthalmologic study of every patient with neurosyphilis, in order to arrive at a presumptive or definite early diagnosis before the development of visual failure, seems essential.

The course of untreated primary optic atrophy is so variable as to render unsafe any conclusions as to the beneficial effects of treatment in the individual patient. The process is practically always bilateral, though one eye may be involved weeks or months before the other. The average time from onset of symptoms to blindness is 2 to 3 years, with extremes ranging from a few weeks to many years. Spontaneous temporary remission may occur, but ultimate blindness in untreated patients is inevitable.

Many detailed studies of the pathology of optic atrophy have been made. They have definitely established only one fact, namely, that the process begins in the marginal fibres of the optic nerve distal to the chiasm. The degenerative process apparently bears no direct relationship to the presence or absence of exudative or inflammatory changes in the overlying or neighboring meninges or in the connective tissue septa of the nerve, or to the presence or absence of *T. pallida* in or close to the visual pathways. Pathologic studies of early optic atrophy are difficult to obtain and are largely lacking. Theories of the development of primary atrophy are discussed, none of which as yet offer a completely satisfactory explanation of its mechanism.

Attempts at the experimental production of syphilitic optic atrophy in laboratory animals have been unsuccessful.

The treatment of primary optic atrophy in the pre-arsphenamine era was unsatisfactory. The introduction of the arsphenamines did not materially improve this situation. Patients treated with an intravenous arsphenamine, bismuth, mercury, and the iodides in any combination and for any length of time appear to become blind about as rapidly as if nothing had been done. Subdural treatment, with arsphenaminized serum, neoarsphenamine or bichloride of mercury dissolved in salt solution or spinal fluid, mercurialized serum, or air, injected intraspinally or intracisternally, has, however, been found to be of some value in the hands of many observers. Various techniques of treatment are described. In general, arrest of the atrophic process or even slight improvement may be accomplished in about 50 per cent of patients with early optic atrophy by the prolonged application of this method of treatment. Patients most suitable for treatment are those in whom the atrophic process is still unilateral, or in whom visual acuity in the better eye is not worse than 20/40.

The results obtainable from subdural treatment are sufficiently encouraging to justify its more widespread and patient trial. That the method is not yet in more general use depends on many factors, perhaps the most important of which are that reports so far published are based on small numbers of patients followed for relatively short periods of time, and that most of them lack convincing ophthalmologic data.

Fever therapy, chiefly with malaria, has also been found by some observers to be of value in arresting the progress of optic atrophy, though these favorable opinions are offset by the unsatisfactory experience of others. As yet, fever therapy is to be regarded as in the experimental stage. The percentage of favorable results obtained from its use is lower than from subdural treatment, and treated patients have been followed for inadequately short periods of time. Malaria has been noted to precipitate sudden visual failure during its course, and is, moreover, attended with some risk to life.

Tryparsamide is absolutely contraindicated in the treatment of the syphilitic optic atrophies.

The choice of a treatment method in the optic atrophies due to syphilis depends on a thorough general study of the patient. The association of other lesions of syphilis in the nervous system or in the body elsewhere than the nervous system, or of complicating diseases, will often be the determining factors. Each patient represents an individual problem calling for expert care and the closest cooperation between ophthalmologist and syphilologist.

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EXPERIMENTAL EPIDEMIOLOGY[1]

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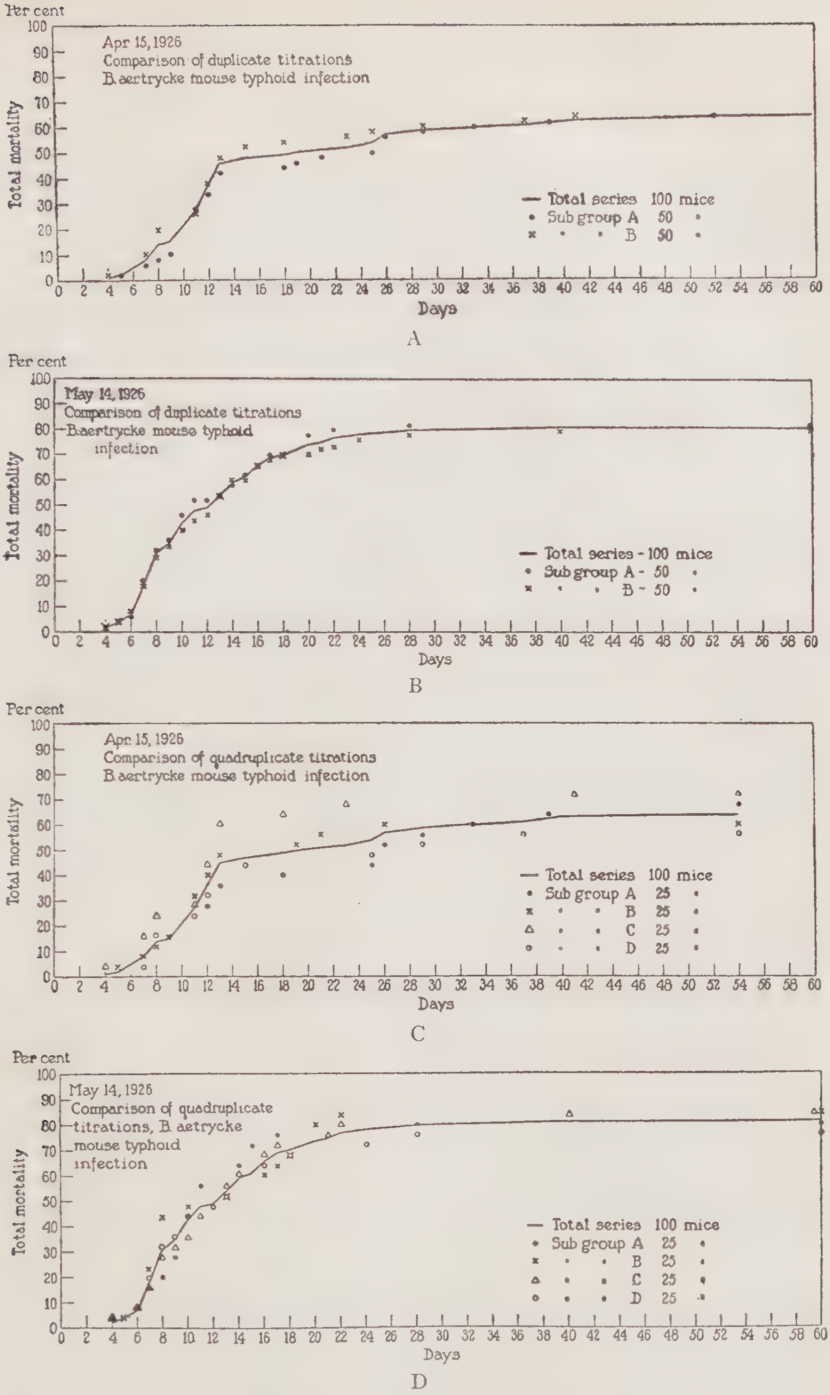
Some intensive experimental work in epidemiology has been in progress during the past twelve years. It was commenced at a time when the theories of communicability and microbic etiology of certain epidemic diseases were widely accepted, when the events of infection were described in terms of microbic virulence, dosage, and host resistance, and when epidemic outbreaks were attributed for the most part to enhancement of virulence and their abatement to loss of virulence and to acquisition of specific resistance by the host. Already these theories were being criticized. In the first place, the discovery of "healthy" carriers indicated that disease and epidemics especially must be due to something more than mere exposure of host to virulent microörganisms. Again the old ideas that the inherent resistance of the host, diet, climate, and season act to influence in some manner the spread of infection were being reinforced by recent observations. Most important, however, was the realization that the old established techniques of epidemiology, bacteriology, and immunology had failed to yield the data necessary to solve the remaining problems of epidemiology. The descriptive epidemiologist, for example, was submitting to mathematical analysis vital statistics containing gross uncontrolled sources of error; the bacteriologist was studying the problem of virulence in artificial infections and artificial media without determining whether his findings are especially related to the behavior of microörganisms in their native host; and the immunologist was gaining knowledge of the specific substances found in the blood of individuals who have been exposed to microörganisms without investigating thoroughly the extent to which these specific substances are related to the resistance of the host under natural conditions. In short, experimental evidence was not forthcoming to answer the question of whether

[1] Harvey Lecture, 1932.

the mass phenomena of infectious disease can be explained in terms of virulence, dosage, and host resistance.

Shortly after the influenza pandemic of 1918–1919, investigations were commenced by Topley (1) in London and by Flexner and Amoss (2) in New York, to bring to light through experiment the general mechanisms underlying the spread of infectious diseases. These studies continued and amplified by others have come to be regarded as a means of attacking directly the present problems of epidemiology. The technique of the method deserves consideration in some detail.

Experimental epidemiology has dealt thus far with bacterial infections of small animals. A total of nine enteric and respiratory diseases of rodents and fowl have been analyzed by observations on population infections occurring spontaneously and induced experimentally, and by measurements of virulence, dosage, and host resistance, during the actual spread of infection. The technical details have differed according to the emphasis which each investigator has placed upon control of variables and adherence to conditions which bring about natural infection. It was assumed at the outset that the method of experimental infection most apt to yield critical evidence on epidemiological problems is one which simulates natural conditions by bringing about the clinical, pathological, and mass phenomena of the natural disease. In the Rockefeller Institute laboratories the specific bacteria are introduced into the native hosts by way of the normal portal of entry and in doses such as might conceivably reach the animal in the natural course of events. In so far as could be determined, the procedure reproduced the phenomena of natural infection closely. The greatest control of variables consistent with natural infection was obviously to be desired since one or many factors might conceivably influence the course of disease. Therefore, in our laboratory every effort was made to regulate the host variables as well as the microbic ones. The animals employed were born and raised in special rooms on the premises. Their heredity, age, and weight have been known, their allotted space, cleaning routine, their food, temperature and light have been kept uniform. Thus it has been possible to be certain that the animals had no previous exposure to the infection studied. The procedures have given results which were quantitative in that populations of animals exposed to an infectious agent under similar conditions



Text-fig. 1. Comparison of Duplicate and Quadruplicate Titrations of Resistance of Rockefeller Institute Strain Mice to *B. aertrycke* Mouse Typhoid

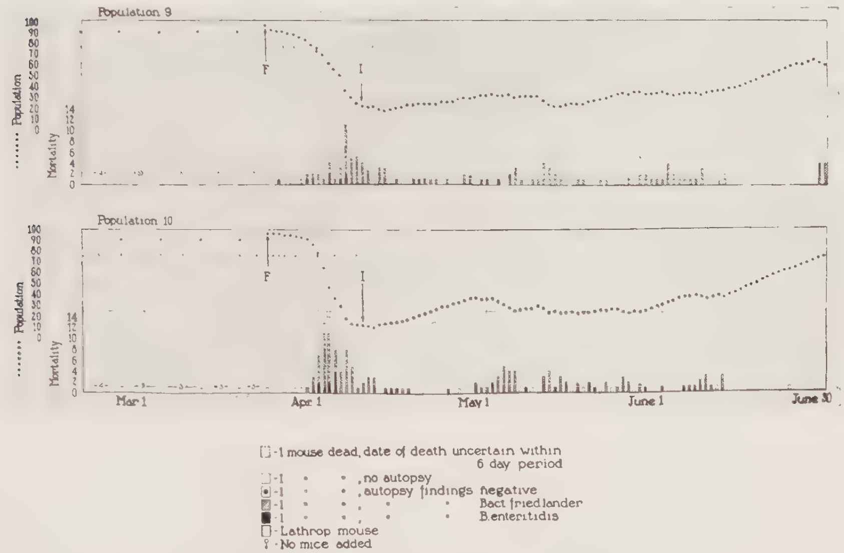
Tests were made on the first day of each month from April to November, 1927. Dose 5,000,000 bacilli instilled into the stomach by tube.

responded in like manner, and that titrations to measure virulence, dosage, and resistance carried out in duplicate and quadruplicate, and repeated at short intervals gave results varying less than 10 per cent (text-fig. 1).

To summarize, a technique has been evolved which is quantitative and yet reproduces the natural phenomena of infection, which permits critical observations on the questions of whether microbic and host factors account for all the mass phenomena of infectious disease, and if so, in what manner these variables function.

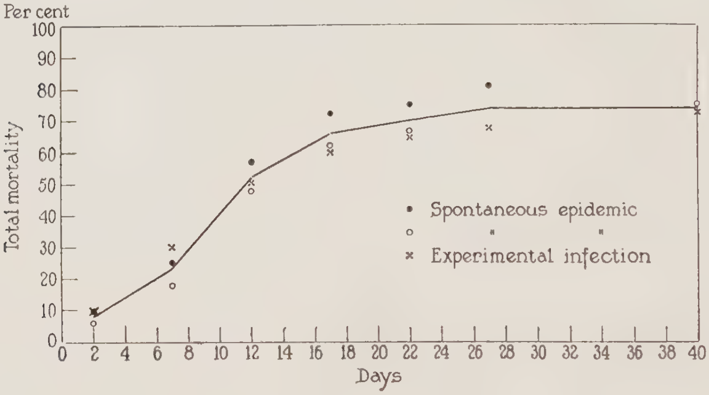
The data provided by the method of experimental epidemiology will be considered with reference to five major findings. One is that all the phenomena of infection studied thus far experimentally can be described completely in terms of microbic and host factors. Another is that microbic virulence, although potentially variable, is under natural conditions relatively stable. A third is that microbic dosage varies with the amount and severity of infection, increasing prior to outbreaks and decreasing prior to subsidence of infection. A fourth finding is that breeds and individuals possess definite and different amounts of non-specific inherited resistance to infection, that this resistance is affected by environmental factors, such as season and diet and may be supplemented by a specific immunity. Finally, it developed that the amount of resistance present in individuals or populations at a given time determines largely the extent and severity of disease. These results will now be considered in detail.

Early experiments showed that natural infection could be established in a susceptible population by administering pure cultures of specific bacteria to constituents or to immigrants. Amoss, in 1922 (3), fed mouse typhoid cultures to ten mice and placed them in two cages in the midst of 100 normal mice in twenty cages. All were fed by the same attendant who went from cage to cage. The specific infection proved fatal to eight of the ten and spread to the normal animals. Later, four batches of mice were added and the infection extended to each group in turn. In our laboratory in 1930 (4) Friedländer pneumonia was established in four populations of mice by administering 500 bacilli intranasally to certain individuals. In one experiment the entire group received the organisms and healthy immigrants were added thereafter at the rate of two per day for three and a half years. In two further experiments three groups of 100 mice each received 6 immigrant mice which had been given 500 Friedländer bacilli intranasally. Healthy immigrants were added thereafter for two and a half years and eight months respectively. In each case the Friedländer infection spread from the immigrants to the healthy population and became established with endemic and epidemic phases identical with those of the spontaneous disease (text-fig. 2).



TEXT-FIG. 2. EPIDEMICS OF *B. friedländeri* PNEUMONIA FOLLOWING INTRODUCTION OF CARRIERS INTO EXPERIMENTALLY CONTROLLED POPULATIONS OF HEALTHY ROCKEFELLER INSTITUTE STRAIN MICE

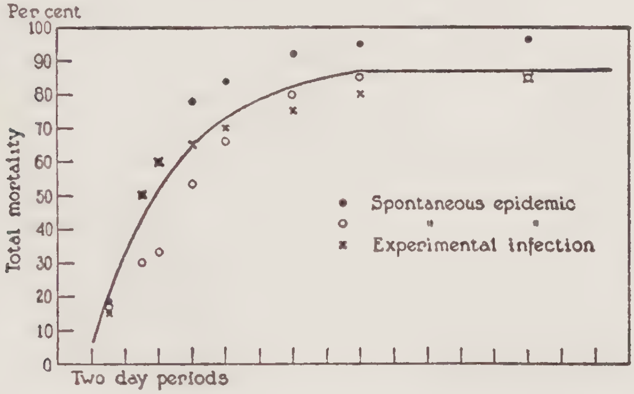
Early experiments likewise showed that epidemics could be induced in populations under two sets of conditions. First, by administering to each individual of previously unexposed communities a certain dose of the specific organisms by way of the natural portal of entry, and second, by adding susceptible immigrants to already infected populations. Epidemics of mouse typhoid were incited in previously unexposed populations by administering three to five million bacilli in broth intrastomachally to batches of 20 to 100 mice. The resulting mortality over a sixty-day period in averaged tests was characteristic and practically identical with that occurring during spontaneous epidemics (text-fig. 3). Epidemics of Friedländer pneumonia were incited in previously unexposed populations by administering 500 bacilli in broth intranasally to batches of 50 to 100 mice. The resulting mortality over a thirty-day period was uniform in averaged tests and similar to that occurring during spontaneous epidemics (text-fig. 4). In infected populations, outbreaks of mouse typhoid were incited by adding batches of normal susceptible immigrants. Following some suggestive observations of Topley in 1919 (1), Amoss assembled 100 healthy mice in twenty cages of 5 mice each and placed in their midst two additional cages of 5 mice each, which had been fed on a culture of mouse typhoid bacilli (5). Four weeks later forty additional cages, containing 5 mice each, were placed next the first batch. Subsequently, sporadic infection occurred, fatal in three months to about 50 per cent of the population. The survivors were recruited to their original numbers by replacements to maintain 5 mice in each cage. Within a few days an epidemic of mouse typhoid occurred, fatal to 70 per cent of the population in two months. A second and a third replacement were made, followed by outbreaks of decreasing severity. These results were confirmed in 1925 by Greenwood and Topley (8), who showed that the repeated addition of normal individuals to mouse populations infected with Pasteurella is followed by recurring epidemic waves. Likewise, experiments at the



TEXT-FIG. 3. COMPARISON OF MORTALITIES IN SPONTANEOUS EPIDEMICS (5) AND EXPERIMENTAL INFECTION (6). *B. aertrycke* MOUSE TYPHOID

Rockefeller Institute in 1930 (4) showed that if immigrants were added to one of three mouse populations infected with *B. friedländeri*, recurrent epidemics of pneumonia ensued, while if at the same time and under identical conditions, immigrations were discontinued in the two remaining populations, no epidemics occurred and the infection died out.

Topley and Greenwood, in further observations on infected mouse populations (8), emphasized the close association between mortality and the number of susceptible immigrants. They found that when mice were added to an infected community at a constant rate, the mortality was propagated in regularly recurrent waves and suggested that the character of the waves was dependent upon the rate of addition of immigrants. We observed later that when four or more populations of mice infected with *B. friedländeri* (4) were recruited at the rate of 2 mice per day, the primary epidemic waves of pneumonia occurred when the population had reached a certain level, lasted a definite number of days, and reduced the number of animals to a definite low level. Moreover, when *B. enteritidis* typhoid was spreading under identical conditions in uncomplicated form in these communities (9), the mortality waves occurred with considerable regularity at nine- to eleven-day intervals. It is plain that epidemics can be



Text-fig. 4. Comparison of Mortalities in Spontaneous Epidemics (4) and Experimental Infection (7). *B. friedländeri* Mouse Pneumonia

Reprinted from *J. Exp. Med.*, 1930, **52**, 918 incited in already infected herds by the addition of susceptible immigrants in suitable numbers, and in unexposed populations by administering to each individual a certain dose of the specific organisms.

Early in these studies, Topley observed that, following epidemics of mouse typhoid, there are a certain number of survivors, and he stated that these survivors are both potentially infective and relatively immune (10). Evidence of the immunity of survivors was obtained later in our laboratory by actually comparing the resistance of surviving and non-exposed mice (11). Mouse typhoid organisms were administered by mouth to 100 individuals; sixty days later there were thirty-two survivors. These, together with 20 normal unexposed mice, were then given a similar dose of bacteria. Sixty days later 30 per cent of the survivor group had succumbed, as compared to 80 per cent of the control group, showing that by actual test, survivors are more resistant than non-exposed animals.

The results thus far described demonstrate that various endemic and epidemic phenomena of natural enteric and respiratory infection can be elicited experimentally solely by bringing together susceptible hosts and pathogenic microörganisms, thus furnishing additional experimental evidence for the infection hypothesis brought forward by the Pasteur-Koch school fifty years ago. Another accomplishment of these experiments has been to afford an opportunity for analyzing the microbic and host factors under natural, yet controlled, conditions, and of determining whether virulence actually varies during epidemics and whether epidemics are brought to an end by the population acquiring a specific immunity.

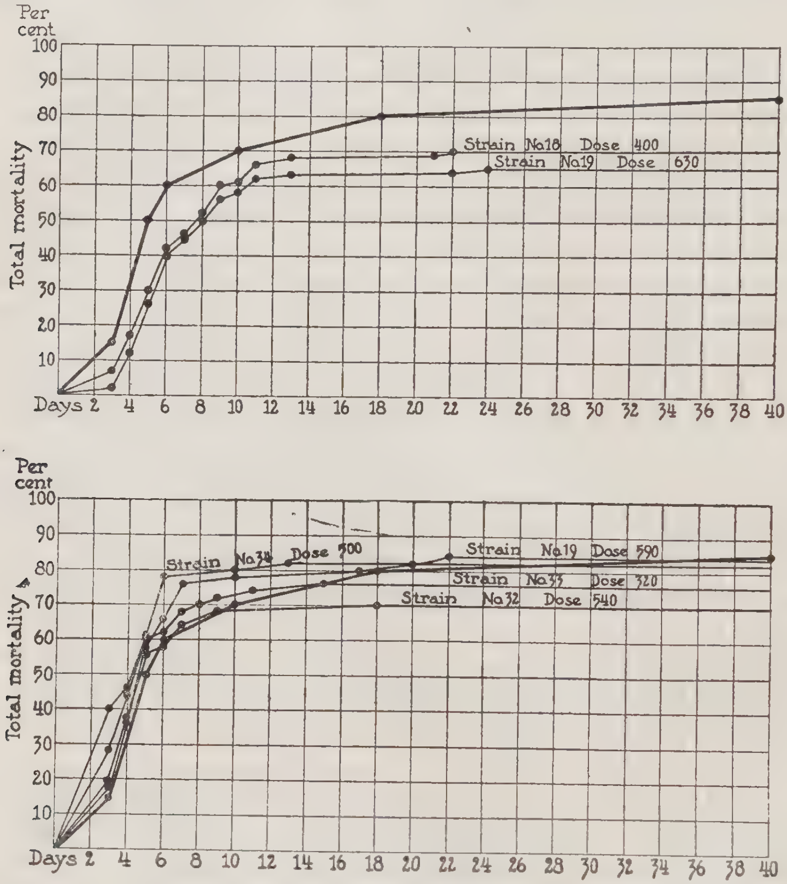
The question of the virulence factor will first be considered. The assumption that the virulence of a microörganism, its capacity to harm its native host, is a highly labile variable is derived from the early observations of Davaine and Pasteur. Supported by later bacteriologists, it has come to form the basis of modern epidemic theory. The experimental epidemiologist, however, recognizing that it is derived from tests which are now regarded as inadequate, has interested himself in the virulence of bacteria under conditions as nearly natural as possible.

In one series of experiments, typical strains of organisms from infected animals were found to be of uniform virulence, whether recovered early or late in the course of disease (12). For example each of two cultures of mouse typhoid organisms were given intrastomachally to 20 mice, one from the heart's blood of an animal dying with acute septicemia five days after exposure to mouse typhoid; the other from the blood of a mouse with chronic septicemia, alive and apparently healthy five weeks after exposure. The acute septicaemic culture proved fatal to fourteen; the chronic septicemic culture to 12 mice.

Other tests showed that strains of mouse typhoid, Friedländer, and fowl cholera organisms from healthy carriers were in general of the same virulence as strains recovered from fatal cases. Thus, each of three strains of *aertrycke* mouse typhoid organisms was given by mouth to 20 mice, one a culture from an acute septicemic case, two others from stools of 2 mice surviving and healthy five weeks after exposure. The mortalities were 70, 70, and 65 per cent, respectively (12). Tests with *B. enteritidis* mouse typhoid gave similar results (9). Several strains of mouse Friedländer organisms were administered intranasally to batches of 50 or 100 mice in comparable doses (4). In one instance, a culture from the lung of a fatal case was compared with a culture obtained from the nasal passages of a healthy carrier. Each was given to 100 mice intranasally. The resulting mortalities were 70 and 65 per cent, respectively. Tests were made with thirty fowl cholera strains from fatal cases and forty strains from healthy carrier fowl on several commercial farms (13). Each strain was administered into the nasal cleft of twenty specially bred young birds. In general, strains from fatal cases proved to be of the same virulence as similar strains from healthy carriers.

Further experiments indicated that strains of specific organisms recovered from a given population at various endemic and epidemic periods of spontaneous infection were of uniform virulence. Epidemics of fowl cholera (13) and rabbit-snuffles pneumonia (14, 15), mouse typhoid (9) and mouse pneumonia (4) (text fig. 5) were studied in the Rockefeller Institute Laboratory, epidemics of guinea pig and rabbit Pasteurella and pneumococcus pneumonia by Neufeld and Freund (16), and epidemics of guinea pig typhoid by Theobald Smith and Nelson (17). In no instance were significant differences in the virulence of pre-epidemic, epidemic, and post-epidemic strains detected.

Still other tests showed that strains of the same organisms from different populations may differ in virulence and in general that the strains with high initial killing potency possessed relatively little ability to persist in the tissues of survivors and vice versa. This latter fact found illustration in experiments in which an epidemic or endemic strain of fowl cholera organisms was administered intranasally to young chickens and permitted to spread to healthy contacts



TEXT-FIG. 5. TITRATIONS OF VIRULENCE OF EPIDEMIC AND ENDEMIC STRAINS OF *B. friedländeri*

Strain 18 Carrier culture, healthy individual, inter-epidemic period. Strain 19 Lung culture, fatal case, epidemic period. Strain 32 Lung culture, fatal case, epidemic period. Strain 33 Lung culture, fatal case, epidemic period. Strain 34 Carrier culture, healthy individual, post-epidemic period.

(18). The tendency of the epidemic strain to kill but not to persist in survivors or spread to contacts, in contrast to the tendency of the endemic strain to persist and spread but not to kill, was consistent. The same differences were demonstrable with epidemic and endemic strains of rabbit Pasteurella (19).

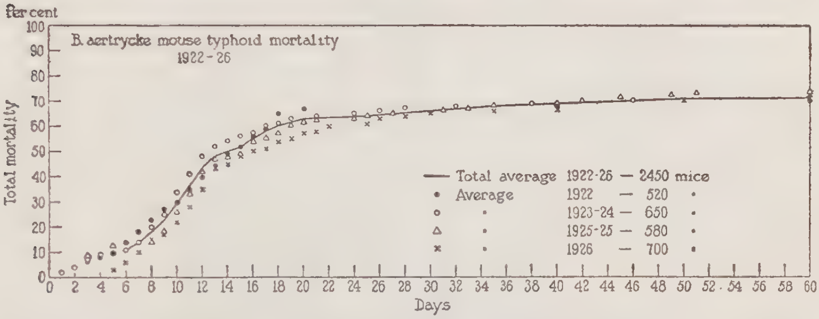
The influence of bacterial dissociation on the prevalence of rabbit and fowl pasteurelloses, mouse *B. enteritidis* and *B. friedländeri* infections has been studied. In the case of rabbit pasteurellosis, virulent "S" strains of *P. lepiseptica* were dissociated *in vitro* into "G" forms of low virulence (20). This was observed *in vivo* by giving rabbits virulent "S" strains intranasally and taking cultures from the nasal passages of survivors (21). From a few individuals, "G" forms were recovered for short periods of time. However, if rabbits of a population in which pasteurellosis was spreading spontaneously were cultured from the nasal passages, "G" forms were seldom, if ever, present. Only the virulent forms spread from animal to animal (22). The organisms of fowl pasteurellosis behaved in a similar manner (13–18). Virulent strains of *B. friedländeri* associated with mouse pneumonia were dissociated *in vitro* into avirulent "G" forms (7). But during three years' observation on the spread of the disease in eight mouse populations, "G" forms were not found on carrier or autopsy cultures. If the "S" forms disappeared from a community, the infection likewise died out (4). Finally, virulent strains of *B. enteritidis* were dissociated *in vitro* by means of a highly potent bacteriophage associated with the spontaneous *B. enteritidis* mouse typhoid infection (23). These "M" and "R" forms, however, neither persisted nor spread when administered *per os* to normal mice (24). And during four years' experience with eight infected mouse populations in which the bacteriophage was present in many autopsy cultures, the dissociated forms were encountered in rare instances and insignificant numbers (9). The virulent forms were responsible for the spread of the disease from host to host. It is concluded, therefore, that microbic dissociation phenomena played no part in determining the prevalence of infections thus far studied.

One further series of tests may be mentioned briefly—namely, those of the effect of animal passage on virulence. Since the early days of bacteriology, cultures have been transferred from animal to animal for the purpose of enhancing their virulence. The tests have been made for the most part in some foreign animal host and by means of some artificial method, such as intraperitoneal injection, and the results, although definite in a few instances, have usually proved equivocal. Nevertheless, they have been invoked to support the idea that prior to an epidemic the virulence of the specific agent increases by means of passage from host to host. The present tests were made under conditions as nearly natural as possible. Mouse typhoid organisms from heart's blood or intestinal contents of animals in the acute stages of infection were given intrastomachally to batches of mice without intermediate cultivation on artificial media. Such passages were made serially and repeatedly, and at intervals titrations were made of the comparative virulences of the passed and unpassed strains (25). Similar tests were made with Pasteurella cultures native to rabbits (26), and fowl cholera organisms native to chickens (27). In no instance was a significant increase in virulence demonstrable.

In summary, the effective virulence of a given strain of microörganisms, when analyzed under natural and controlled conditions, has proved to be a relatively stable property and in general inversely related to its ability to survive in the tissues of its natural host. The virulence of strains of the same organism in the same community was uniform, while the virulence of strains from different communities was at times dissimilar.

The next epidemic factor to be analyzed was that of microbic dosage —the number of organisms available to the individual host or to the total population at a given time. The findings may be summarized as follows. Changes in dosage exerted a direct effect upon mortality, when virulence and resistance were constant. As dosage was increased, there ensued a progressive increase in percentage mortality up to a point less than 100 per cent. Further increase in dosage had no effect on mortality. Thus, in a titration of mouse Friedländer bacilli, each of eight doses of the organisms given intranasally to 25 mice resulted in mortalities of 32, 44, 56, 64, 88, 96, and 96 per cent, respectively (7). The second finding has been referred to before, namely, that by giving mice proper doses of typhoid or Friedländer organisms, it is possible to reproduce closely the events of explosive, spontaneous epidemics (text-fig. 4). Finally, as will be pointed out in tests referred to later, during the actual spread of infection in populations, dosage increased prior to epidemics and decreased prior to their decline by a time interval which approximated that of the incubation period of the infection (4–9).

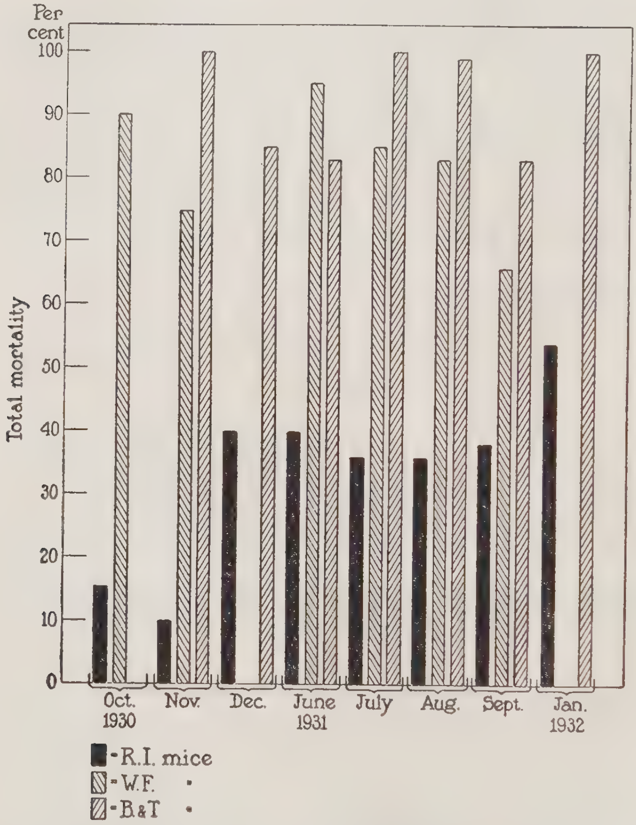
Host resistance, the third epidemic factor, was found to be made up of both non-specific and specific components. The non-specific component was present in certain definite amounts in breeds or races of animals. For example, monthly tests of the resistance of an inbred strain of mice to mouse typhoid organisms were made from 1922 to 1926. The results in terms of mortality were averaged each year and found to approximate each other within 2 or 3 per cent (text-fig. 6).



Text-fig. 6. Comparison of Averaged Monthly Tests of Resistance of Rockefeller Institute Strain Mice to Intrastomachal Instillation of 5,000,000 *B. aertrycke* Mouse Typhoid Organisms

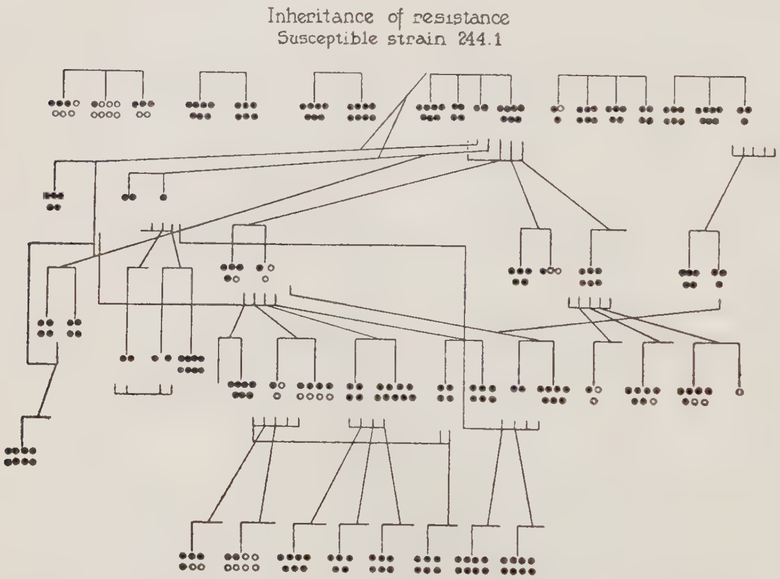
Similar tests with other enteric infections and with respiratory ones, and tests with other strains of mice gave equally consistent results. The non-specific components of resistance were present in different amounts in different races or breeds. Thus, a black Lathrop strain, pen inbred for twelve generations, proved on nineteen consecutive monthly tests with *B. aertrycke* (28) and several consecutive tests with *B. enteritidis* mouse typhoid (9) to be more susceptible than the Rockefeller Institute strain of mice. Albino mice of Swiss strain, also highly inbred, and albino Rockefeller Institute mice were raised on four different diets. In each case the Swiss mice proved more susceptible. Repeated tests with white-faced (text-fig. 7) and black and tan strains, brother and sister inbred, showed them to be more susceptible than the Rockefeller Institute line.

The non-specific component of resistance was also proved to be present in different amounts in individuals of the same breed: Thus, when a controlled group of animals were all given the same, suitable dose of $HgCl_2$, individuals showed markedly different types of response, ranging from death after the briefest interval to a completely refractory state (29). In the same way, when a group of animals previously unexposed were given a native infection by the normal portal of entry, using the same dose of microörganisms, individuals showed markedly different types of response, ranging from death



TEXT-FIG. 7. COMPARATIVE MORTALITIES OF ROCKEFELLER INSTITUTE, WHITE-FACE, AND BLACK-AND-TAN STRAIN MICE TO INTRASTOMACHAL INSTILLATION OF 5,000,000 *B. enteritidis* MOUSE TYPHOID ORGANISMS after the briefest incubation period to a completely refractory state. These differences were more regular and predictable when every precaution had been taken to insure uniformity of inheritance, environmental conditions, and food. In *B. aertrycke* mouse typhoid, for example, when groups of mice were given bacilli intrastomachally, 70 per cent died with positive blood cultures in a period of five to sixty days, while 30 per cent survived. Of these latter, about 20 per cent were recovered cases, with or without positive agglutinins, while 10 per cent showed no evidence of infection (12). Again, when rabbits were given a similar dose of Pasteurella intranasally, approximately 28 per cent died of pneumonia, of acute interstitial, lobar, or chronic empyema types, 44 per cent showed merely local rhinitis or sinusitis, 13 per cent became healthy carriers, and 14 per cent remained uninfected (19, 30). Similar individual differences were manifest after the administration of fowl cholera organisms to chickens. These findings have been confirmed by workers in Neufeld's laboratory in their studies of Friedländer, Pasteurella, and pneumococcus infections of mice (31). In short, individuals submitted to precisely the same risk of infection, under identical conditions, with every known factor controlled, exhibit profound differences in response.

That these differences in the reaction of individuals are due to a non-specific resistance which is inherited was shown by the results of tests on survivors and by breeding experiments. Mice surviving an intrastomachal instillation of mouse typhoid organisms proved more resistant to an instillation of mercury bichloride than unselected mice of the same strain (11). The progeny of mice surviving *aertrycke* or *enteritidis* typhoid were more resistant to subsequent infection with mouse typhoid bacilli or to doses of mercury bichloride than normal, unselected mice of the same strain; and conversely, progeny of mice, succumbing early to typhoid, were more susceptible than unselected mice (32). A further study of the inheritance of different degrees of susceptibility is now in progress. Five hundred female and 100 male mice of the Rockefeller Institute strain were mated, 1 male to 5 females. When the young were weaned, the 600 parents were given intrastomachally three million *enteritidis* mouse typhoid bacilli. In cases in which both parents died within ten days after infection and in which, in contrast, both parents survived sixty days, the respective litters from susceptible and resistant parents were saved for further breeding. Simultaneous tests of resistance have since been made on the progeny of six generations of the original susceptible and resistant groups together with an unselected control group. The possibility of specific immunity developing from infected animals was definitely ruled out by testing the population for carriers, by testing animals found dead for mouse typhoid organisms, and by housing the progeny of susceptible and resistant lines together in the same cage for four weeks. The stock has remained free from all infection. Thus far, five lines of susceptible mice and six lines of resistant mice have been selected. The susceptible lines show approximately 95 per cent mortality within fifteen days after exposure (text-fig. 8); the control group shows 35 to 40 per cent, and the resistant lines show approxi-

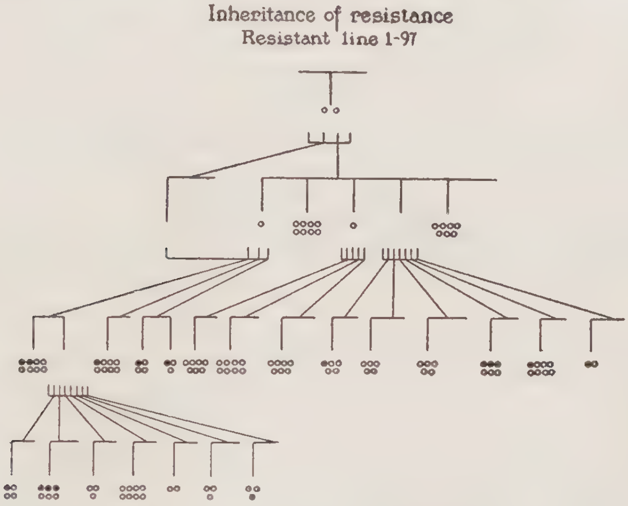


Text-fig. 8. Effect of Selective Breeding on Resistance of Rockefeller Institute Strain Mice to *B. enteritidis* Mouse Typhoid

Each circle represents a tested mouse; each group of circles, a tested litter. A solid black circle signifies that the mouse died of mouse typhoid following instillation of the organisms; an open circle, that the mouse survived.

Susceptible line no. 244-1.

mately 5 per cent mortality over the sixty-day period of observation (text-fig. 9). The same differences in resistance are still manifest when the resistant mice are given ten times the dose and the susceptible mice $\frac{1}{100}$ of the standard dose. The crucial test of the resistance of these mice has been their response to exposure to spontaneous infection. Cages were arranged to contain 5 unselected mice given five million *B. enteritidis* by mouth, together with 5 normal mice from the susceptible lines and 5 from the resistant lines. In each instance, all or nearly all of the contact susceptible mice contracted the infection and died, while all or nearly all of the resistant mice survived and remained healthy. Finally, it should be said that the resistant lines prove more refractory than the susceptible lines to fowl Pasteurella and pneumococci instilled intranasally. These results prove that of 500 individuals selected at random, some possess a greater and some a less amount of resistance which is transmitted quantitatively to their progeny.

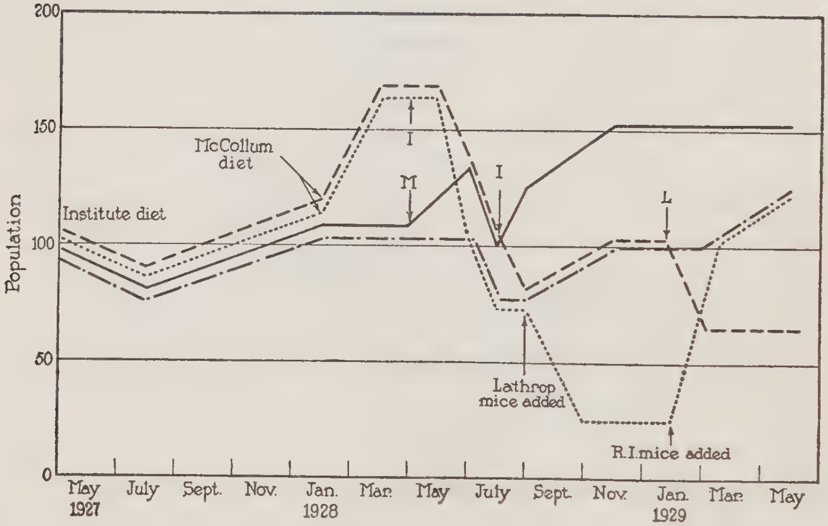


TEXT-FIG. 9. EFFECT OF SELECTIVE BREEDING ON RESISTANCE OF ROCKEFELLER INSTITUTE STRAIN MICE TO *B. enteritidis* MOUSE TYPHOID

Each circle represents a tested mouse; each group of circles, a tested litter. A solid black circle signifies that the mouse died of mouse typhoid following instillation of the organisms; an open circle, that the mouse survived.

Resistant line no. 1-97.

Non-specific components of resistance, besides being inherited, are affected by environmental factors such as season and diet. Thus, each month over a period of years, batches of 50 to 100 mice have been given mouse typhoid or Friedländer organisms with the result that mortalities, although irregular, have been definitely greater during the winter than the summer seasons (33). Again, mice raised on various adequate diets differ markedly in resistance. One diet containing bread and milk has sufficed to raise the Rockefeller Institute stock for more than fifteen years. The fertility, duration of life, weight, appearance, and general health of these animals are excellent and have become standard. Another diet used by McCollum has proved adequate for his rodent stock, and a third, a modified Steenbock ration, has likewise proved satisfactory. And yet, mice of the same strain, raised on the bread and milk diet, are far more susceptible to *enteritidis* and *aertrycke* mouse typhoid, botulinus toxin, and mercury bichloride poisoning than mice fed on McCollum or Steenbock formulae. No intensive efforts have been made thus far to



TEXT-FIG. 10. EFFECT OF CHANGING HOST RESISTANCE ON THE SPREAD OF *B. enteritidis* MOUSE TYPHOID INFECTION IN MOUSE POPULATIONS HOUSED IN SINGLE CAGES

The chart pictures events in 4 populations for 2 years in terms of census figures analyze these differences beyond demonstrating that butter fat and cod liver oil added to the bread and milk changed the "susceptible" into a relatively "resistant" diet (34). Environmental factors, other than those tested, such as exercise, fatigue, and exposure probably exert some influence on the resistance mechanism of the host, but as yet have not been submitted to adequate examination.

The known specific components of resistance appear to be acquired as a result of contact with the specific agent. Just how potent these specific components may be and how important epidemiologically has not been determined. Perhaps the most complete test of these questions has been reported by Topley and Greenwood (35). Animals were vaccinated with killed cultures of *B. aertrycke* and exposed to infection by being placed in an infected population. The amount of protection conferred was claimed to be significant only when a certain type of flagellar vaccine was employed, and then only when the exposure was mild and of brief duration. The protection was not a permanent one; it was noted during a period of about ten to thirty days after exposure.

In sum, the analyses of host resistance to infection have shown that individual animals exhibit definite amounts of non-specific inherited resistance to primary infection which take the form of a frequency distribution characteristic of the breed or race. Moreover, this resistance may be added to or reduced by such environmental influences as season and diet, and may be supplemented by specific immunity components acquired as a result of contact with the specific microbic agent.

The effect of the resistance factor on the spread of infection has proved to be important. In the first place, it was demonstrated that the form of explosive epidemic curves, that is the time distribution of morbidity or mortality, is an expression of individual differences in the resistance of population constituents to a fixed dose of organisms of known virulence. As shown previously, when a number of mice are each given an equal number of bacteria of a single culture, or even a like dose of $HgCl_2$, they succumb after varying periods which when plotted take the form of an epidemic curve similar to that of spontaneously occurring epidemics (text-fig. 3 and 4). In the case of the experimentally treated mice, individual differences in resistance are the only variables which can account for the time distribution of mortality; and in the case of spontaneous epidemics the same is probably true.

A second effect of the resistance factor is to control to a great extent the prevalence of endemic infection. This relation, together with that of virulence and dosage, in actually inciting epidemics has been determined most effectively in studies of experimentally controlled epidemics of fowl (18), rabbit (26), and mouse Pasteurella (36) and mouse Friedländer (4) and *enteritidis* infections (9). These studies, in furnishing critical evidence on the questions at issue, may be regarded as typical of experimental epidemiology. The results with the mouse populations exemplifying the findings as a whole will alone be considered.

Mice constituting a population were assembled in a single large cage and maintained on the routine diet of bread and milk. Two normal mice with identification marks were added daily. A census was taken each day and the animals found dead were removed, autopsied, and cultured for the presence of the specific organism.

The first experiment made was designed to test the mode of spread of Pasteurella in communities previously unexposed to these organisms (36). Populations 1, 3, and 4 received rabbit strains; Population 2 a fowl strain. The three rabbit strains infected a few of the mice and died out at once; the fowl strain behaved in quite a different manner. Eight of the 10 mice originally fed died within two weeks with Pasteurella septicaemia, after which no further deaths from this infection occurred for six weeks. At that time, however, one of the immigrants succumbed. Three weeks later, when the population numbered 61 individuals, an explosive epidemic of Pasteurellosis broke out, fatal to 77 per cent of the population in five days. The epidemic then ceased abruptly and Pasteurella disappeared from the community. The virulence of six strains obtained during the epidemic proved to be uniform and similar to that of the culture originally introduced into the community.

The next experiments dealt with explosive and highly fatal epidemics of Friedländer pneumonia which arose spontaneously in the mouse populations (4). In the communities to which daily immigrations of 2 mice were continued, characteristic secondary waves ensued. These waves appeared when the population number reached a certain level. They lasted a relatively similar number of days and reduced the population to a similar low number. Subsequently, endemic periods intervened, followed by the disappearance of the disease in the late winter. During the next summer, epidemics broke out again similar in each population but more protracted than the previous ones. Fewer secondary waves ensued and the disease disappeared sooner. The virulence of the Friedländer organisms, as determined by their ability to spread and incite typical epidemics in populations of previously unexposed mice, and by titrations of strains obtained during pre-epidemic, epidemic, post-epidemic, and inter-epidemic phases of the infection, proved to be constant. The carrier rate increased prior to epidemic outbreaks and decreased shortly before the time of peak mortality. A substitution of relatively susceptible for standard immigrants in a given population was followed by an increase in severity and frequency of the epidemics, while on the other hand, substitution of resistant for susceptible immigrants was followed by a decrease in the severity of the infection.

The final experiments were made with *enteritidis* mouse typhoid in the same mouse populations (9). The infection took the form of periods of low grade mortality, interspersed with epidemiform outbreaks. The daily deaths in the one case were either relatively constant or rhythmic in nine-day intervals; the sudden increases in mortality were invariably associated with some definite environmental disturbance. Cultures of the organisms taken from healthy carrier or mice dead of typhoid during pre-epidemic, epidemic, or inter-epidemic phases of infection were of uniform pathogenicity. Furthermore, the bacterial dissociation and bacteriophage phenomena, although abundantly present, seemed to play no part in determining the spread of infection. The dosage of the organisms available to the population increased just prior to an increase in death rate and decreased in like manner before a fall in death rate. Most important were the experiments made to test the effect on the prevalence of infection of changing population resistance. Increasing population resistance by substituting an optimum for a barely adequate diet or by substituting relatively resistant for susceptible immigrants each day inaugurated periods of relatively low death rate; on the other hand, a depression of population resistance by substituting the barely adequate diet for the optimum one, or susceptible for resistant immigrants, was followed by severe epidemic outbreaks.

This concludes the present findings in experimental epidemiology. It has been noted that various phenomena of population infection can be reproduced experimentally merely by the bringing together of host and microbic factors under suitable conditions. In these infections, spontaneous or experimentally induced, strains of microörganisms from different populations differed occasionally in virulence—the more virulent being the less vegetative—but the effective virulence of strains in any one community proved stable during the entire endemic and epidemic periods of observation. The dosage and host resistance factors, on the contrary, varied significantly with the amount and severity of infection. Expressing these relationships in terms of cause and effect, it appears that the severity of infections in these animal populations was regulated by stable virulence and varying dosage and resistance factors. In instances in which a foreign microorganism gained access to a hitherto unexposed population, the inherent virulence, the available dosage, and the amount and distribution of non-specific population resistance together determined the extent and severity of the infection. In instances in which a microorganism was already present in the population, variations in population resistance and in available dosage were chiefly responsible for endemic and epidemic prevalences. Fluctuations in population resistance were brought about by immigration, season, and diet acting upon the non-specific components, and by the infecting agent stimulating the specific components of resistance. Fluctuations in available dosage resulted from variations in the host resistance and vector factors.

To what extent is this experimental knowledge consistent with the known facts of human epidemiology? Briefly, there is evidence that the factors related to microbe and host suffice to account for the usual manifestations of cholera, typhoid, and the insect and animal-borne infections. There are no data indicating that these factors do not suffice in other human infections. Concerning the operation of these factors, there are grounds supporting the view that infections transmitted by vectors or contracted from foreign hosts, and infections transmitted by water, milk, or food are for the most part controlled by a fluctuating dosage factor operating on a population of fluctuating resistance. These diseases, taken together and considered from the time-space viewpoint, constitute the great majority of the total number. Added to them are the parasitic and skin infections, the prevalence of which appears likewise to be controlled by the host and dosage factors. The remaining group, that of respiratory diseases transmitted by direct contact, relatively very small but frequent in temperate climates, and therefore of great interest, are at present not as well understood. One can only say that the available data do not contradict the view that their prevalence is controlled by fluctuations in the dosage of and resistance to specific agents of relatively fixed virulence.

Further knowledge of the spread of human infections is now being sought by analyses similar to those utilized in experimental epidemiology. Opie's studies on the spread of tuberculosis in families (37), Paul's observations of families with rheumatic disease (38), the work on the spread of upper respiratory tract pathogens among small groups of individuals (39), and detailed bacteriological, clinical, and sociological investigations of circumscribed communities, throw light on the manner and extent of dissemination of the specific agents and the relation of variations in dissemination, that is, in dosage, to variations in amount and severity of the infection in these communities. They promise to make more clear the rôle of resistance in disease and the relative importance of its non-specific and specific factors.

To broaden the scope of experimental epidemiology the studies must be extended from the acute, highly fatal bacterial diseases of animals to the more chronic ones and to virus infections. This last step is already being taken (40). Knowledge of many types of infection in many species of hosts will be required for the proper development of epidemiology as a science.

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FACTORS CONCERNED IN THE EVACUATION OF THE GALL BLADDER[1, 2]

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Of all the hollow viscera, the gall bladder has been the most baffling in regard to the mechanism of evacuation. In fact, a few investigators (1–4) have expressed the view that under normal conditions, the gall bladder does not evacuate its contents through the cystic duct. However, most physiologists since the time of Haller have taught that the primary function of the gall bladder is to deliver concentrated bile to the duodenum at the beginning of digestion.

Some of the difficulties which have been responsible for considerable disagreement in regard to the mechanisms concerned in the evacuation of the gall bladder are as follows: (a) The gall bladder possesses but little smooth muscle when compared to such hollow viscera as the stomach, intestine, or urinary bladder,—a fact that has engendered considerable skepticism in regard to the contractile power of this organ. (b) On direct mechanical or electrical stimulation, the gall bladder manifests relatively little or no evidence of contractility when observed under the conditions present during the usual laboratory and operating room procedures. (c) In order to obtain evidence on the contraction processes in the gall bladder, considerable care and attention must be given to the depth of the anesthesia, blood pressure level, operative trauma, state of digestion, and method of registration of movements; in addition, technical skill and experience are required in certain phases of such a study. (d) Because the gall bladder normally absorbs certain of the biliary constituents, notably water, and other substances introduced into its lumen, and may in the course

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[1] The meaning of the term "evacuation of the gall bladder" is limited in this lecture to the exit of gall-bladder contents via the cystic duct.

[2] Lecture delivered before the Harvey Society, New York, February 18, 1932.

of time under certain abnormal conditions (obstruction of the cystic duct) absorb most, if not all of the biliary constituents, one must consider the possibility that under normal conditions contents may leave the gall bladder only by being absorbed.

The contractile power of the gall bladder. Galen (5), in the second century, found from his dissections that the gall bladder is "sometimes very full, sometimes empty, and sometimes in an intermediate state," and taught that the gall bladder fills and empties like the other hollow viscera. Haller (6) in 1755 taught on the basis of experimental evidence that the gall bladder contracts, but not to the extent of the urinary bladder, a fact that has been denied and reaffirmed by subsequent observers. Doyon (7) in 1893 made the first graphic records of gall-bladder contractions. Since then, such records have been obtained by every investigator who has used adequate methods. Genuine contractions have been recorded from the gall bladder *in situ* in both anesthetized and unanesthetized animals, chiefly in the dog, and from "strips" of the gall-bladder wall.

The motility of the gall bladder (8–14) in the dog is of two types: first, a *tonic contraction* of the musculature which causes a prolonged increase in intra-gall-bladder pressure lasting from five to thirty minutes or more; and second, a *tonus rhythm* in which the gall bladder contracts and relaxes at a rate of from two to six times per minute. In each type the musculature as a whole or a portion of it may contract. With careful technique, a spontaneous tonus rhythm may be observed in 60 per cent of dogs or even in gall-bladder "strips." The tonic contraction may lead to a maximum rise in intra-gall-bladder pressure of from 20 to 30 cm. of bile with the cystic duct obstructed (5 to 7 cc. of bile being displaced from the gall bladder into pressure recording tubes). The changes in pressure produced by the tonus rhythm vary from 0.1 to 3.0 cm. of bile. At the height of the tonic contraction, the tonus rhythm usually disappears, and then reappears during the relaxation phase of the tonic contraction. A sub-threshold stimulus for a tonic contraction may augment the rate and amplitude of the tonus rhythm without causing a general increase in tone. A mild distension of the gall bladder may result in slight tonic contraction. Prolonged mild distention usually causes a compensatory relaxation. Ravdin (15) has recently observed rhythmic contractions of the gall bladder of man *in situ* and in "strips" from the human gall bladder. Some contractility of the gall bladder has been observed in every animal studied.

Does the gall bladder evacuate? It is established and generally agreed that the gall bladder contracts, but opinion has not been unanimous as to whether the musculature prevents distention, facilitates absorption, or causes the expulsion of bile. I suspect that it performs all three functions, since the smooth muscle of the intestine performs the same general functions. However, before answering the question as to whether the gall bladder evacuates through a contraction of its musculature, it must be shown that it evacuates its contents through the cystic duct under normal conditions.

Much indirect evidence (16) was reported during the period from 1885, when Heidenhain did his work on the secretion of bile, to 1924, when Graham and Cole visualized the gall bladder, which indicated that the gall bladder was the source of the dark, viscous bile that flowed from the common bile duct after certain procedures. During the latter part of this period, the stimulating idea of Meltzer (17) of the reciprocal relation of the gall bladder and the sphincter of the common duct was recorded. On this idea were based the provocative observations on "non-surgical drainage of the gall bladder" of Lyon (18). Much credit is due to Meltzer and Lyon for re-awakening the interest of the medical world in regard to gall-bladder physiology. Probably the most direct attacks on the problem during this period were those of Auster and Crohn (19), Winkelstein (20) and others (21, 33a) who placed substances in the gall bladder to determine their time and mode of explusion. The discovery that the gall bladder can be visualized by tetraiodophenolphthalein by Graham and Cole (22) in 1924, gave us a new method for the study of gall-bladder physiology in animals and man. The discovery of Boyden (23) that egg yolk will cause bile to disappear from the gall bladder of the cat, and by Sosman, Whitaker and Edson (24) that most fats have a marked effect on the disappearance of the cholecystographic shadow in man provided physiologists and clinicians with an important and a potent excitant of gall-bladder activity. Shortly after these discoveries, Whitaker (25) showed that the cat's gall bladder filled with iodized oil is evacuated through the cystic duct after the oral administration of fatty substances; McMaster and Elman (26) by the method of "altercursive intubation" demonstrated the expulsion of a portion of the gall-bladder contents by contraction of the viscus after feeding; and Higgins and Mann (27) under direct vision observed the gall bladder of guinea pigs and dogs to contract and evacuate through the cystic duct, a fact that has been confirmed for the guinea pig by Burget and Brocklehurst (28) and for the dog by Lueth, Ivy and Kloster (14), and by Ivy, Sacks and Drewyer (29) who have made moving pictures of the phenomenon. So from direct evidence, it is established that the gall bladder of the cat, dog and guinea pig during contraction may evacuate its contents through the cystic duct.

Evidence has been obtained by Halpert and Hanke (30) in the rabbit which indicates that the rabbit's gall bladder does not evacuate through the cystic duct, although using the same method they found that the dog's gall bladder evacuates completely (31) each day via the cystic duct. We have been inclined to agree with their observations on rabbits, because on using the ordinary tambour and manometer technique, we have not been able to obtain definite contractions of the gall bladder of the rabbit (14). However, Dr. Walsh and I (32) have demonstrated recently by direct observation and photography of the gall bladder and by chemical analysis of the duodenal contents that the gall bladder of the rabbit may evacuate under the stimulation of "cholecystokinin," from 60 to 80 per cent emptying being obtained. The contractions were pseudo-peristaltic in nature, usually starting at the neck as Higgins (33) described in the bullhead (fish), although the contractions might start at both ends and meet in the middle or start in the middle and move towards both ends. Evacuation occurs either with an intact common-duct sphincter or with the common duct cannulated and the hepatic ducts tied. We could not obtain rapid evacuation of iodized oil in the rabbit; the shadow disappeared gradually but not completely throughout an observation period of five days. We did observe marked changes in contour of the gall bladder analogous to those observed under direct vision and photography. Evidently the iodized oil was too viscous to be evacuated by the relatively feeble contractions of the rabbit's gall bladder. Babkin and Webster (33a) have observed evacuation of the rabbit's gall bladder after egg yolk and cream were given by stomach tube.

Unequivocal evidence that the gall bladder of man may evacuate via the cystic duct is also available. This evidence is of three kinds, surgical, chemical and roentgenological.

Four surgeons have reported that they have seen the gall bladder empty on duodenal stimulation in patients with the abdomen open. Sachs (34) of Nebraska reported one case with the abdomen open in which a distended gall bladder started to collapse five minutes after instilling magnesium sulphate (50 cc., 33 per cent) into the duodenum. An apparent contraction was not seen. Pribram (35) of Austria reported a case in which the gall bladder emptied after instillation of a peptone solution (20 cc., 10 per cent), active contraction being observed. Matsuo (36) of Japan exposed the gall bladder under pantapon-scopolamine and local anesthesia in two patients. In one case the instillation of magnesium sulphate into the duodenum was effective in evacuating three-fourths of the gall bladder contents within thirty-five minutes. Then the vesicle was partially filled with azorubin S, after which magnesium sulphate was again instilled and the dye obtained from the duodenal drainage. The gall bladder was seen to contract. The dye test was repeated on the second case with the same results except that the gall bladder was not seen to contract. Trommer and Hempel (37) have reported five cases in which India ink was placed in the gall bladder at operation and then obtained from the duodenal drainage after instilling 60 per cent glucose into the duodenum. They did not look for evidence of contraction.

Chemical evidence bearing on the question of whether the human gall bladder evacuates has been obtained by analysing the duodenal contents for iodine, bile pigment, and cholesterol before and after the instillation of magnesium sulphate, N/20 HCl, and oleic acid solutions into the duodenum. In the experiments in which an analysis for iodine concentration was made, the gall bladder was previously visualized with tetraiodophenolphthalein. In such experiments performed by Lake (38) and by Lyon (18), the results indicate that the gall bladder evacuated a considerable portion of its high iodine-containing bile. Mann and Higgins (27) obtained similar results in the dog. However, Sweet (1) has interpreted these results as indicating the duodenal stimulation caused the iodine to be absorbed rapidly by the gall-bladder mucosa and then re-excreted by the liver from whence it passed into the duodenum. Such a view is hardly tenable on the basis of the rapid change in the gall-bladder shadow that so frequently occurs after the ingestion of egg yolk and fat, and in view of the slow rate of absorption of tetraiodophenolphthalein by the gall bladder as observed in the experiments of Sweet (1) (39a) and Johnson (39), and in view of the fact that some normal subjects show very little change in the gall-bladder shadow on starvation or on eating several carbohydrate meals within twenty-four hours (24) (49) (41). Of course, it is possible that duodenal stimulation with fat increases the rate of gall-bladder absorption, but Johnson (39) reports that a fat meal does not increase the rate of absorption of the dye.

Adlersberg and Taubenhaus (42), using the Meltzer-Lyon method, have reported a three to ten-fold increase in bilirubin concentration of the duodenal contents after the instillation of magnesium sulphate. This has been confirmed by Jones and Walsh (43) in my laboratory for normal subjects; they found cholesterol also to be present in some samples of duodenal contents in the concentration in which it is present in gall-bladder bile, and since absorption by the "normal epithelium" of the bile ducts does not occur according to all available evidence, the only known source of such bile is the gall bladder.

The cholecystographic evidence that the human gall bladder evacuates is now conclusive (23) (44). Much cholecystographic evidence has been obtained by Boyden and others (41) (24) (45) from human subjects showing marked changes in the contour and size of the gall bladder within ten or fifteen minutes (against gravity, subject standing) after the application of various procedures which are known to cause the gall bladder of animals to contract and evacuate. In order to account for some of these changes on the basis of the absorption of dye, absorption would have to occur in the gall bladder more rapidly than it occurs in a loop of intestine of equal size. The most striking evidence, however, is that by using the proper radiographic technique, the cystic and common bile ducts become visualized after giving a fat meal to subjects with a normal cholecystogram. Boyden (44) and Pendergrass (46) have made such observations. I have seen numerous such films as shown in figures 1, A, B, and C, in the department of Dr. James T. Case, who states that either the cystic duct, common duct, or a portion of the hepatic duct, or all, become visible after the

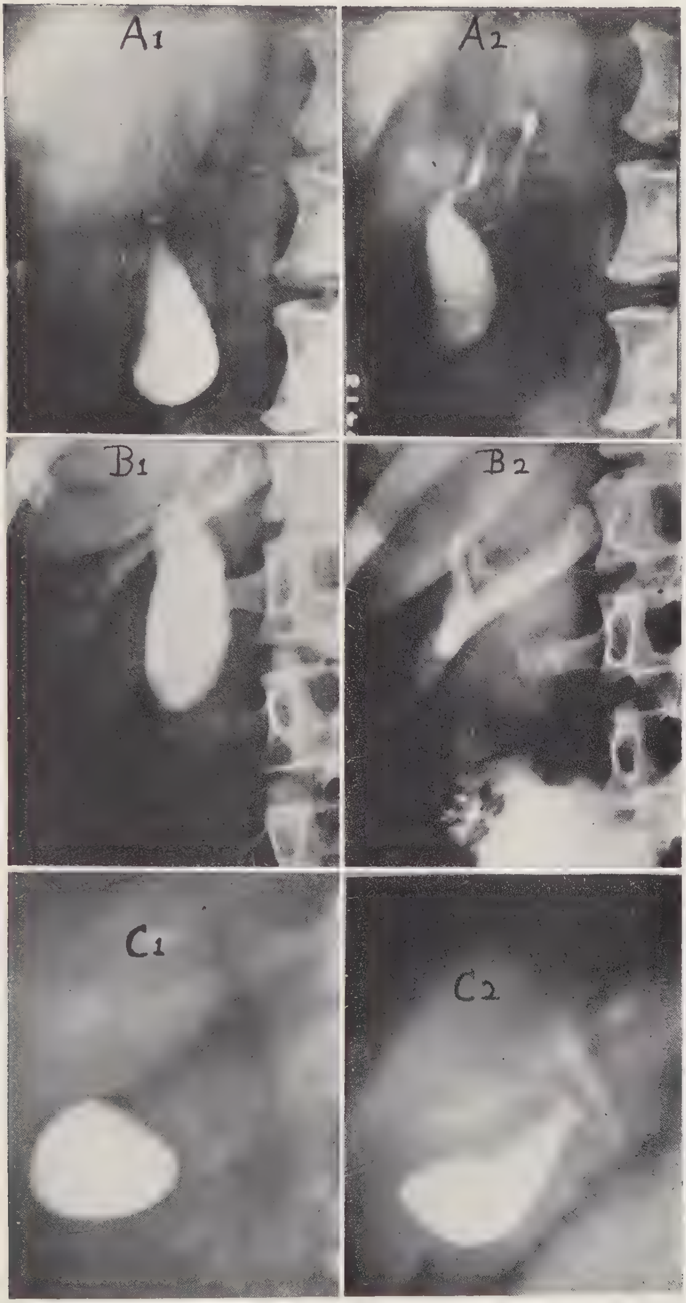

FIG. 1. HUMAN CHOLECYSTOGRAMS BEFORE AND AFTER A FAT MEAL

The pictures A, B and C were taken fourteen hours after the intravenous injection of the dye. Then a fat meal was ingested and the pictures A_2, B_2 and C_2 were taken 20 min. later. In A_2 and B_2 note cystic and common ducts. In C_2 note cystic, common and hepatic ducts.

fat meal in about 10 per cent of patients with a normal cholecystogram. The ducts may become visible within 15 or 20 minutes after egg yolk. Such roentgenograms cannot be explained on the basis of absorption of the dye from the gall bladder and re-excretion by the liver. If so, the hepatic ducts should be as visible as the common and cystic ducts and in those cases in which the hepatic ducts become visible after the ingestion of egg yolk and cream, there should not be a clear cut line of demarcation between visibility and invisibility, as is seen in figure 1, C_2. This and the foregoing evidence conclusively demonstrate that the gall bladder of man evacuates under normal conditions via the cystic duct.

Since the gall bladder of the cat, dog and man while contracting may evacuate via the cystic duct, the obvious deduction is that its musculature plays an important rôle in the evacuation process. The pressure exerted on the contents of the gall bladder during a maximal contraction has not been measured in man; in the dog a maximal pressure of from 20 to 30 cm. of bile is exerted,—a pressure that is not to be considered as trivial. However, before one can be assured of the sufficiency of the above deduction, one must consider (a) the mechanisms concerned in the activation of the gall bladder musculature, and (b) the possibility that evacuation through the cystic duct may be due in part to other factors than contraction of the gall-bladder musculature.

Mechanisms concerned in gall-bladder contraction. The following causal mechanisms for gall-bladder contraction have been suggested: (1) local stimulation by distention or by "pungent" bile, (2) nervous reflex stimulation from various portions of the gastro-intestinal tract, (3) long nervous reflexes from the higher brain centers and, (4) humoral agents, including the hormone, cholecystokinin. As is true for other hollow viscera, all may be concerned.

Local stimulation. It is interesting that Galen (5) states, "For since it was shown that the gall bladder attracts its own special juice so as to be often found full, and that it discharges it soon after, this desire to discharge must be either due to the fact that it is burdened by the quantity or that bile has changed in quality to pungent and acrid." No direct experiments have been done on the possibility that the gall bladder may be caused to discharge its contents in response to an adequate tension stimulus or irritation of the mucosa by the contents. The facts that the gall-bladder wall contains ganglia, that spontaneous contraction or evacuation has been observed, that distention of the isolated viscus will cause a slight contraction, and that a certain optimum distention and pressure are required for a maximum response to a stimulus, indicate that local stimulation of the gall-bladder wall may be a factor. I should be surprised if this were not true. There is indirect evidence to the contrary, however. This topic deserves further investigation.

Nervous reflex stimulation from various portions of the gastro-intestinal tract. Since the various portions of the gastro-intestinal tract may be reflexly inhibited or excited by stimulation of distant parts, the same might be expected to be true of the gall bladder. Boyden and Birch (47) have obtained evidence showing that electrical excitation of the pyloric antrum frequently induces a slight contraction, and that the electrical excitation of the intestine and particularly the cecum induces relaxation of the gall bladder. That the gall bladder may be inhibited reflexly is of obvious clinical significance. But there are no good experimental or clinical data bearing on the problem of a relation between gall bladder emptying and enteritis, colitis or constipation.

Meltzer's theory (17) that a reciprocal innervation exists between the gall bladder and the common-duct sphincter is sound physiologic philosophy. According to this theory gall-bladder contraction reflexly causes relaxation of the sphincter—not the reverse. In spite of the fact that there is much evidence pro and con, the evidence most free from technical objections (48) (49) shows that when the gall bladder contracts forcibly, the resistance to the flow of bile into the duodenum decreases. However, it is known that the gall bladder may contract against a contracted "sphincter" (13) (23). There is no crucial evidence at hand proving the theory. One is not surprised at the disagreement in the literature in view of the multiplicity of factors, the slight pressure changes observed, and the variation in response of the sphincter and gall bladder from time to time in the same animal. There is even disagreement in regard to the reciprocal relation of the stomach to its sphincters, which is best explained by the observations of Carlson and Litt (50) that the muscular response obtained frequently depends on the physiological state of the neuromuscular mechanism at the time the stimulus is applied. That the mechanism of reciprocal innervation is not essential for gall-bladder evacuation is shown by much crucial evidence to be cited later.

Long nervous reflexes from higher brain centers. Although a number of investigators (7) (8) (9) (12) have obtained motor effects in the gall bladder on excitation of the vagi and splanchnic nerves, none has claimed that the changes are marked. Appreciable emptying has not been obtained by vagal stimulation in unanesthetized dogs (25) (40). Some (26) (49) (52) have observed what might be termed a "psychic" contraction of the gall bladder with the expulsion of from 1 to 3 cc. of bile on the sight or taste of food or the aroma of bacon. Some report failures (51) (53) (40). Crandall (40) has shown that "sham feeding" of egg yolk and meat for from one-half to one hour in the dog, the gastric juice being removed, does not cause an apparent change in the size and density of the gall bladder visualized with tetraiodophenolphthalein. This does not mean that a "psychic" contraction of the gall bladder does not occur, especially in view of the well known quantitative variation of "psychic" secretion of gastric juice in man and dog. The disagreement only indicates that the response is slight and variable.

Humoral agents. Two different types of humoral agents (54) are known to be concerned in causing gastric secretion: secretagogues, which are present in food or arise from the digestion of food and act both locally and by being absorbed into the blood; and a hormone, histamine-like in nature, which has a specific effect on the gastric glands. By analogy, it is possible that the gall bladder may be caused to contract following the ingestion of a fatty or protein meal, either by the absorbed fatty substances, or by a hormone produced by the action of fatty substances or acid chyme on the gastro-intestinal mucosa.

This possibility occurred to a number of investigators at about the same time, particularly Boyden (23), Whitaker (25), Copher and Illingworth (55) and others (11), because Whitaker had obtained contraction and evacuation of the "denervated" gall bladder and McMaster and Elman had made a similar observation with the cystic duct cut and intubated. Boyden tried blood transfusion with suggesis the most potent intravenous excitant of gall-bladder contraction known at present.

It has been shown that the rise in intra-gall-bladder pressure caused by cholecystokinin is not due to extraneous factors such as increase in liver volume (fig. 3), diaphragmatic changes, and gastric or intestinal motility (14). The gall bladder of the dog and rabbit has been seen by direct vision and photography to contract and evacuate after an injection.

A large dose of atropine (2–10 mgm.) decreases, but does not abolish the response. Ergotamine (1.5 mgm.) which *per se* causes a slight contraction of the gall bladder, does not decrease, but tends to increase, the response. These observations indicate that the active principle acts directly on the smooth muscle (14) (59).

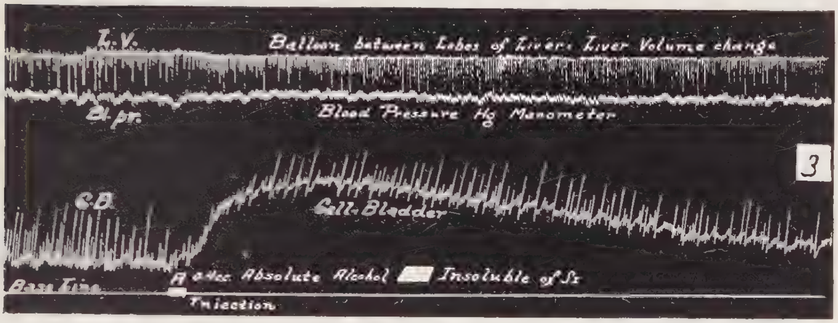

Fig. 3. This tracing shows that the rise in gall-bladder pressure is not due to a change in liver volume. Cholecystokinin has no detectible effect on liver volume. Note that the pancreas in this case did not respond. (Reprinted by permission of the American Journal of Physiology, **91**: 331, 1929.)

The active principle in the doses employed had a variable effect on intestinal motility, sometimes increasing, at other times decreasing or having no effect (14).

Since we have made preparations which caused gall-bladder contraction and not pancreatic secretion and vice versa, we believe that the gall-bladder contracting principle is different from secretin (60). We have, therefore, proposed the term "cholecystokinin" to designate the gall-bladder contracting principle of extracts of the upper intestinal mucosa. Cholecystokinin and secretin are closely related, and on occasion we have observed the apparent conversion of one into the other. But the question of identity will not be settled until one or the other or both have been crystallized.

Two observers (62) (63) have reported contraction of gall-bladder "strips" or of the isolated organ on the introduction of "secretin" solutions into the bath. One observer (61) failed. Jung (66) has found that the isolated gall bladder of the guinea pig in a "bath" is very susceptible to change in pH. However, when cholecystokinin is added, taking precautions not to disturb pH, a contraction results. From five to ten intravenous dog doses must be added to the bath, probably because cholecystokinin diffuses very slowly through the gall-bladder serosa.

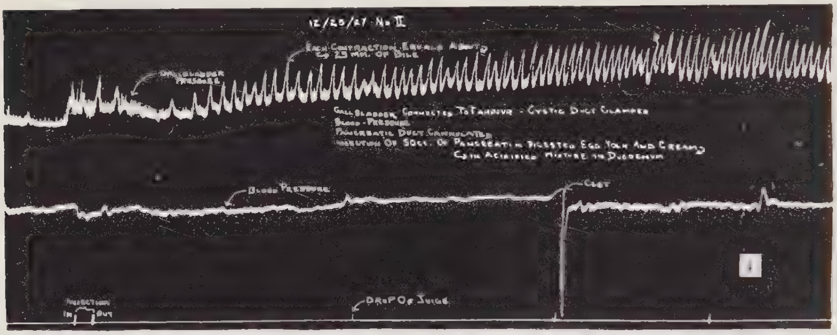

Fig. 4. The upper tracing shows the change in gall-bladder pressure after the introduction of a slightly acidified pancreatic digest of egg yolk and cream into the duodenum. A slowly increasing tonic contraction of the gall bladder as a whole is to be noted on which is superimposed a tonus rhythm augmented in rate and amplitude. Each tonus rhythm exerted a pressure of 2.5 cm. of bile on the contents, and they occurred at a frequency of from two to three per minute. (Reprinted by permission of the American Journal of Physiology, **86**: 605, 1928.)

It has been found that when either hydrochloric acid of the strength that is found normally in the stomach or egg yolk, cream, and olive oil digested with pancreatin are introduced into the duodenum, a contraction of the gall bladder is induced (fig. 4). The contraction begins in about two minutes. Following the introduction of emulsified and partially digested fat, the gall bladder manifests a slow tonic contraction on which is superimposed an augmented tonus rhythm. This, we believe, represents the normal type of gall-bladder response to ingested egg yolk and fat and is well calculated to expel contents from the gall bladder.

The observation that the introduction of dilute HCl (N/10–N/20) into the duodenum caused the gall bladder to contract but that a reasonable intravenous injection of the acid did not, made it possible to test out a hormone hypothesis for gall-bladder contraction. Accordingly cross-circulation experiments were done using dogs with compatible bloods. It was found that the introduction of acid into the duodenum of one dog caused the gall bladder of this dog to contract within two minutes and the gall bladder of the other in from eight to twelve minutes (13). The obvious interpretation of this result (fig. 5), which was obtained in three of four experiments, is that a hormone mechanism is concerned in gall-bladder contraction.

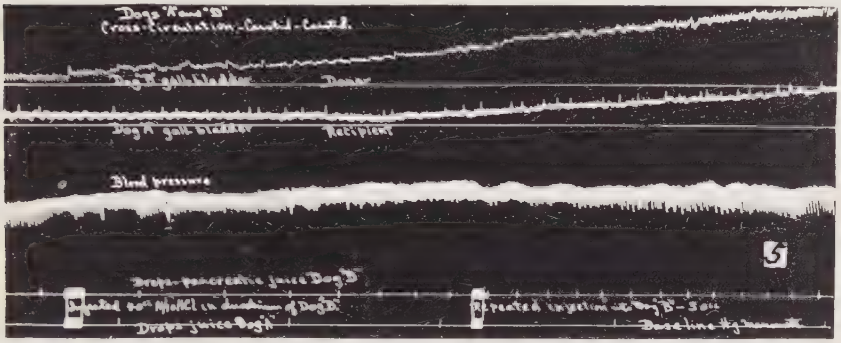

FIG. 5. This tracing demonstrates the result of a cross-circulation experiment. N/10 HCl was placed in the duodenum of Dog B with the result that B's gall bladder contracted within a minute and A's gall bladder contracted about ten minutes later. (Reprinted by permission of the American Journal of Physiology, **86**: 605, 1928.)

Although no other excitant of cholecystokinin production than dilute hydrochloric acid was used in our cross-circulation experiments, we do not believe that acid is the sole excitant. Just as there are other excitants of secretin production than acid, there are probably other excitants of cholecystokinin production. Acid was chosen for our experiments because it gave a rapid response and is a pure chemical substance. We have shown that filling of the gall bladder occurs in gastrectomized dogs and evacuation is induced by feeding egg yolk. Also, perfusion of egg yolk through the stomach in some dogs with a pouch of the entire stomach induces evacuation of the gall bladder (56) (67). Small quantities of cholecystokinin have been obtained from the pyloric mucosa (68).

Cholecystokinin injected intravenously has been observed to cause evacuation of iodized oil from the gall bladder of the dog, of normal gall-bladder contents in the dog, and rabbit, and to cause the Graham-Cole shadow of the gall bladder in dog (fig. 6) and man (fig. 7) to disappear rapidly. Cholecystokinin works more rapidly, but no more effectively than a meal of egg yolk, cream and olive oil. This has been confirmed by Whitaker (59). The amount of evacuation obtained within one hour by successive injections of cholecystokinin varies from

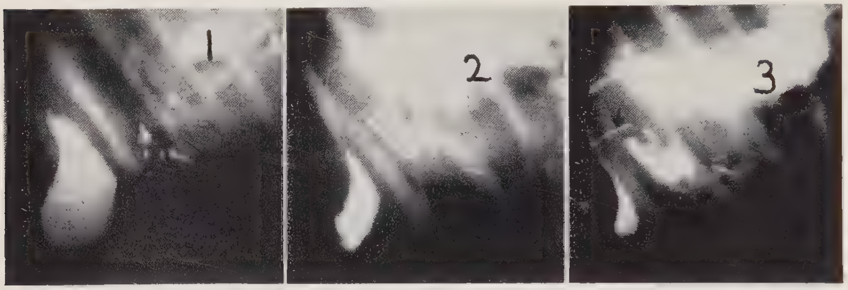

FIG. 6. This figure demonstrates the evacuation of iodized oil from the gall bladder under the influence of cholecystokinin. "1" is the control picture; "2" was taken at 30 minutes; and "3" at one hour. Seven injections of cholecystokinin were made at 10-minute intervals. In "3" note the injection of the hepatic duct of the right lower lobe of the liver. (Reprinted by permission of the American Journal of Physiology, **86**: 605, 1928.)

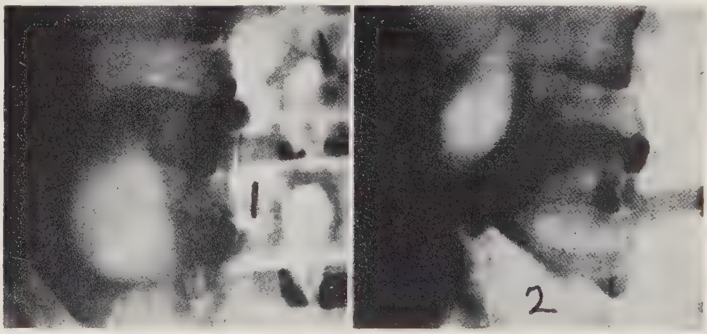

FIG. 7. THE GALL BLADDER OF A PATIENT (MAN) BEFORE (1) AND AFTER (2) THE INJECTION OF THREE DOSES OF CHOLECYSTOKININ AT 10-MINUTE INTERVALS

The changes in the gall-bladder shadow become evident within five or ten minutes of the first injection. The second picture was taken about 45 minutes after the first. (Reprinted by permission of Endocrinology, **14**: 343, 1930.)

tive results. Whitaker, and Copher and Illingworth and others (20) (11) tried "secretin" with negative results; Braga and Campos (64) and Brugsch and Horsters (65) obtained positive results. Whitaker injected emulsified olive oil intravenously with positive results; but since emulsified liquid petrolatum (56) produced the same effect, he questioned the physiologic nature of the response. Higgins and Wilhelmj (57) obtained no evacuation on injecting various emulsified fats intravenously, and Silverman and Dennis (58) observed evacuation in man without a significant change in blood fat. We have found that egg yolk intravenously does not cause gall-bladder contraction. Emulsified olive oil causes an increase in gall-bladder pressure which is an artifact due to marked splanchnic congestion. Chyle has been injected with negative results, and Boyden has obtained evacuation after a fat meal with the thoracic duct tied near the cysterna. Hence, post-absorptive circulating fatty substances appear not to be responsible for gall-bladder contraction and evacuation.

Hormone mechanism. In 1926, being impressed by the observations just mentioned, Dysart and I were successful in transplanting the gall bladder into the omentum, but could not obtain a contraction from the transplant, probably because it was imbedded in an omental mass.

At that time we were engaged in an attempt to isolate the hormone "secretin" for the purpose of perfecting a human pancreatic function test. It occurred to me that since fats are potent excitants of pancreatic secretion and since bile and pancreatic juice play such an important rôle in fat digestion, the hormone "secretin" might cause the gall bladder to contract. Having a number of different "secretin preparations" on hand, Dr. Oldberg and I undertook to assay them on the gall bladder of the cat, and found that some of them caused the vesicle to contract. This was duplicated on the dog, on which animal most of our work has been done.

The method used for recording changes in gall-bladder pressure is one well known to physiologists and is shown in figure 2. By cannulating the cystic duct aseptically under ether anesthesia and then permitting the animal to recover, changes in gall-bladder pressure were followed on unanesthetized animals. It was found that solutions containing as little solid material as 3 mgm., on intravenous injection would cause from 1.0 to 11.5 cm. rise in intra-gall-bladder pressure which persisted from ten minutes to one hour. A series of injections would cause a step-like rise in pressure amounting finally to from 20 to 30 cm. of bile, a pressure which is obtained in the dog after feeding egg yolk or fat (26) (27). Recently we have obtained definite responses with as little as 0.2 mgm. of solid material, and slight changes with as little as 0.06 mgm. Though we have injected many drugs and chemical substances, a response similar to that obtained with the highly "purified" acid extracts of upper intestinal mucosa has not been observed. Acetyl choline (23) (59) and pilocarpine cause a gall-bladder contraction, but these substances affect blood pressure and are antagonized by atropine. Relatively large doses of adrenalin intravenously, but not subcutaneously (23), may cause some contraction (41). The active principle of extracts of upper intestinal mucosa

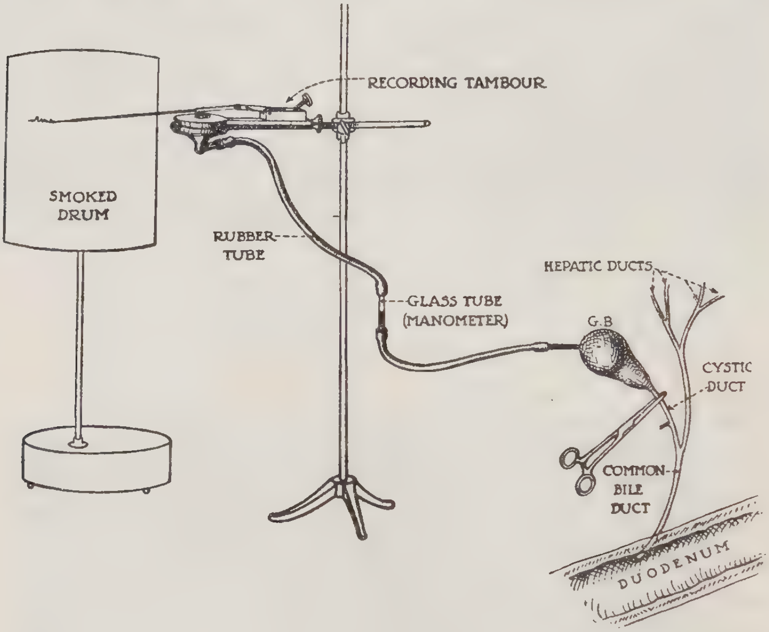

Fig. 2. This figure shows the apparatus and method used for recording gall-bladder contractions. The clamp on the cystic duct frequently occluded the cystic artery and some of the lymphatics. Edema of the gall bladder did not occur. As a rule better contractions following cholecystokinin were obtained when it was not necessary to occlude the cystic artery. (Reprinted by permission of the Detroit Proceedings of the Inter-State Post Graduate Medical Association of North America, October 21–25, 1929.

animal to animal, just as occurs when a fat meal is ingested. We have obtained from thirty to eighty per cent evacuation of iodized oil in one hour. In man we have obtained 50 per cent reduction of the shadow in one-half hour after three injections of cholecystokinin (45).

On the basis of the evidence at hand it is held that a hormone mechanism is concerned in gall-bladder contraction and evacuation. The hormone (cholecystokinin) is closely related to but probably not identical with secretin, and is formed by the upper intestinal mucosa when in contact with acids, and probably fatty and other substances that cause secretin formation.

Spontaneous contraction. Almost every recent investigator in this field has occasionally observed spontaneous contraction and evacuation of the gall bladder in the dog, the cat and man. They have resulted under conditions that preclude a definite interpretation; and hence various interpretations have been offered, none of which is acceptable in fact.

Factors other than contraction which may be concerned in evacuation via the cystic duct. There are some investigators of gall-bladder physiology who are convinced that this organ evacuates through the cystic duct but maintain that evacuation is accomplished primarily by forces other than intrinsic contraction of the gall-bladder musculature. This difference of opinion has led to the performance of numerous ingenious experiments by Winkelstein (20), McMaster and Elman (26), Whitaker (25), Burget (28) (69), Boyden (23), Mann, Potter and Higgins (27) (48) and Graham, Kodama, Copher and Illingworth (22). In regard to the source of the extrinsic forces, the following suggestions have been made: (1) Increase in intra-abdominal pressure incident to inspiration with a relaxed common duct sphincter squeezes bile from the gall bladder into the duodenum. (2) The filled stomach with gastric and duodenal peristalsis pushes the gall bladder against the liver and mechanically massages the bile from the gall bladder. (3) The ebb and flow of bile with the elasticity of the gall bladder wall and a siphonage effect wash out and drain the bile from the vesicle. (4) The milking action of duodenal peristalsis and changes in duodenal tone assisted by changes in intra-abdominal pressure and the elasticity and tone of the gall-bladder wall produce a passive evacuation of the organ. (5) Peristalsis of the biliary ducts in those animals (guinea pig, pigeon, bullhead fish) in which it occurs may play a rôle in emptying the gall bladder. On the basis of the evidence presented in support of these extrinsic forces, I believe there is an element of fact involved in each instance. In order to evaluate these extrinsic factors, it is necessary, first, to determine if the gall bladder will evacuate in their absence, and second, if it does, how these extrinsic factors modify gall-bladder evacuation.

It is definitely established that in the absence of extrinsic forces the intrinsic musculature of the gall bladder in the dog is sufficient to expel most of the contents from the vesicle. McMaster and Elman (26) inserted a tube into the cystic duct, another into the upper end of the common duct, and a third into the lower end of the common duct. By connecting these tubes bile could flow from the liver into the gall bladder or into the duodenum. In this manner the gall bladder was excluded from the flushing action of hepatic bile and the sucking action of duodenal peristalsis. When the gall bladder was filled with bile, it was found that when the animal was fed, a considerable amount of bile was forced from the gall bladder against pressure (20 cm. bile). This cannot be accounted for by an increase in intra-abdominal pressure since the amount of food ingested causes no increase in intra-abdominal pressure according to the experimental data of Cannon (70) and Kelling (71). Higgins and Mann (27) by inserting a tube in the common bile duct and tying the hepatic ducts with the abdomen open, observed evacuation of the gall bladder to occur on introducing a fat meal into the duodenum. No bile left the gall bladder until the fat was introduced, even though the common-duct sphincter control had been removed. Copher and Illingworth (55) filled the gall bladder with iodized oil and placed a cannula attached to a rubber balloon into the cystic duct. Following an egg yolk meal the gall bladder emptied a considerable amount of the oil into the rubber bag.

The rôle of intra-abdominal pressure as affected by deep respiration and vomiting in promoting evacuation of the gall bladder has been stressed by Winkelstein (20). He properly points out that the common-duct sphincter must be relaxed to observe the phenomenon. It is true that deep respirations or vomiting may induce changes in intravesical pressure as high as 10 cm. of bile pressure. Normal respirations, however, with or without the abdomen closed, rarely cause a fluctuation of more than 1 or 2 cm. of bile pressure. When the gall bladder is filled with iodized oil, a very viscous substance, vomiting or marked changes in intra-abdominal pressure artificially induced usually causes no evacuation (24, 25, 26, 55, 75). I have observed slight evacuation of the gall bladder during vomiting when the gall bladder is filled with iodized oil and the sphincter held open by a cannula. Almost complete evacuation occurred after a fat meal. According to some (20) (22), deep manual pressure in the upper right quadrant will squeeze some bile from the gall bladder, but others (24) have not observed this even after atropine. The reason for the discrepancy probably lies in the tone of the common duct sphincter or duodenal musculature. Hence, if respiration and normal changes in intra-abdominal pressure play a minor rôle, their effectiveness depends entirely on the common-duct sphincter.

There is no clear cut evidence supporting the suggestion that the peristalsis of the stomach and duodenum during digestion mechanically massages the bile from the gall bladder (89). That this factor is not even likely is shown by numerous observations to the effect that a carbohydrate meal has little or no effect on the disappearance of the cholecystographic shadow in man.

Graham and coworkers (22) (55) have stressed the possibility that the ebb and flow of fresh bile from the liver with the elastic recoil of the gall-bladder wall may exert a washing out effect. Copher, Kodama and Graham (22) visualized the gall bladder and then tied the hepatic ducts in dogs. A fat meal failed to cause disappearance of the shadow. Scott and Whitaker (78), however, in a similar experiment in cats, obtained emptying of iodized oil (55). The only directly unchallenged experiment in support of this possibility is the following experiment. The gall bladder was removed and a rubber bag was substituted. Tetraiodophenolphthalein was administered and the rubber bag filled and visualized. In five days the shadow had practically disappeared. Whitaker (25) performed the same experiment using iodized oil instead of iodized bile and observed no emptying of the iodized oil after feeding. The results of these experiments indicate that extrinsic forces such as the ebb and flow of bile and the sucking action of duodenal peristalsis or changes in the common-duct sphincter are capable of very slowly evacuating iodized bile, but not the more viscous iodized oil. Boyden (44) has shown that the gall bladder of man will empty against gravity. It is interesting that at one time gravity was supposed to be a factor in emptying of the stomach (70). In view of the evidence cited above on the intrinsic ability of the gall bladder to evacuate, only a minor rôle may be ascribed to the washing out effect of the ebb and flow of bile.

In any consideration of the motor activity of the gall bladder, serious attention must be given to the common-duct sphincter and duodenal motility. All investigators agree that either the common-duct sphincter or duodenal activity or both play an important rôle in the filling of the gall bladder. Most agree that they play some rôle in the evacuation process.

I thought that duodenal peristalsis was the prime factor in gall-bladder evacuation until the experiments of McMaster and Elman and Higgins and Mann were published; and I doubted until recently the existence physiologically of a special common-duct sphincter. This opinion was taught because of the disagreement in the literature in regard to whether the flow of bile into the duodenum was controlled entirely by duodenal tone and motility or by a special sphincter. Recent work on the dog by Lueth (72) in our laboratory shows that to explain the observed facts a correlation of both views is necessary. His graphic records show that "the vagi exercise a tonic motor control over the musculature surrounding the intramural portion of the common bile duct, which may or may not be correlated with changes in tone of the duodenal musculature." This confirms Oddi's original contention. When one inserts a small cannula through the common duct to the level of the submucosal layer and then exerts pressure, the ampulla may be seen to bulge and the pressure must be raised to an adequate threshold before fluid flows into the duodenum. The sphincter of the papilla exerts a pressure of from 2 to 13 cm. of H_2O or from one-third to one-sixth of the total resistance of the intramural portion of the common bile duct. Graphic records were obtained showing a decreased intramural resistance with duodenal relaxation, which confirm the observations of Elman and McMaster (49), Copher and Kodama (73), Burget (69) and others (74). Apparently then the intramural portion of the common bile duct possesses a special sphincteric mechanism which is intimately coördinated with duodenal peristalsis but which may function independently. This interpretation of the facts agrees with the observation of Giordano and Mann (74) who report that bile may spurt from the papillary orifice without discernible peristalsis, but if peristaltic waves are present in the duodenum, the outflow will occur with the peristalses (74a).

Even though duodenal and sphincteric activity may be evident while the gall bladder is evacuating, it does not follow that the activity of these parts is the cause of the evacuation. Berg and Jobling (77) have divided the common duct and implanted it into another portion of the duodenum and obtained filling and evacuation of the gall bladder. As pointed out above, considerable peristaltic activity of the duodenum of man and dog (Higgins has observed the same in the fish) may occur without evacuation of the gall bladder. When the sphincteric resistance in the dog is abolished by section (25) (51), or by a large glass cannula (74) (75) (76) (40) placed in the intramural portion of the common duct, more or less spontaneous emptying occurs within six to eighteen hours. Then almost complete emptying is induced by fat feeding. This early spontaneous emptying is to be expected since the pressure in the gall bladder during fasting frequently amounts to from 4 to 8 cm. of bile (13) and the resistance offered by the sphincter of the common duct may amount to from 20 to 30 cm. of bile. According to Whitaker (25), if the gall bladder of the cat is only partly filled with iodized oil and the sphincter cut, no emptying occurs until fat is fed. These experiments indicate that with the sphincter abolished, the elastic recoil and tonus of the musculature of the gall bladder expel only a portion of the bile. They are not free from objection in that viscous iodized oil was used and in the experiments in which iodized bile has been used, the section of the sphincter may have been followed by edema. These objections, however, are fully met by the experiments of Higgins and Mann cited above, in which the hepatic ducts were ligated and the common duct cannulated and no bile flowed from the gall bladder until fat was introduced into the duodenum. We must therefore conclude that the common-duct sphincter and duodenal musculature assist gall-bladder evacuation by relaxing and thereby permitting bile to be expelled by the contracting gall bladder, and that the "elastic recoil," "the ebb and flow of bile," and sudden changes in intra-abdominal pressure are minor factors.

The common-duct sphincter may inhibit gall-bladder evacuation. The common-duct sphincter and duodenal tone play an important rôle in inhibiting gall-bladder evacuation via the cystic duct. The fact that the intramural portion of the common duct in the dog may exert a resistance of from 0 to 75 cm. of bile under various conditions and that the gall bladder exerts only a maximal pressure of from 20 to 30 cm., shows that in the presence of high intramural resistance, the gall bladder may contract without evacuating. This accounts for the observation that after fat or cholecystokinin, the hepatic ducts in the cat, dog and man are sometimes visualized. Thus, the gall bladder may contract against a contracted sphincter (13) (23) (79). This fact, first suggested by Meltzer, is of considerable pathologic importance, since it has been shown experimentally that chemical duodenitis (80) and duodenal stasis (81) (82) delay the rate of emptying of the gall bladder.

It is an interesting fact that the maximal power of the gall bladder to contract is approximately the same as the secretory pressure of bile and that both are greater than the "average" common-duct sphincteric pressure in the "intact" dog.

Effect of gastro-intestinal operations on gall-bladder evacuation. It is worthy of note that various gastro-intestinal operations, such as gastrectomy (56) (67), gastro-enterostomy (24) (25), pyloroplasty of various types (83) do not affect the response of the gall bladder to a fat meal. The same is true if the stomach is anastomosed to the jejunum and the duodenal secretions emptied into the ileum (83), which should be expected to occur on either a hormone or reflex basis.

Sphincter at the neck of the gall bladder. A possible factor concerned in filling and evacuation of the gall bladder which deserves more attention than it has received is that a sphincter may be located at the junction of the gall bladder and cystic duct. Certain observations made by Volborth (84) may be explained on this basis, and Mann (85) (48) has obtained differences in pressure between the gall bladder and cystic duct which are best accounted for by a sphincter at the neck of the gall bladder. This report deserves further careful investigation. (The author omits intentionally a discussion of the valves of Heister because the diverse opinions of their functional activity are based primarily on anatomic theory (1) (16) (86).)

Gall bladder evacuation in cholecystitis. It is important to know from a clinical and physiological view point whether the acutely inflamed gall bladder will evacuate. If an acutely inflamed gall bladder does not evacuate, this would indicate that the prime mechanism for evacuation lies within the vesicle. Graham (22) has written that "shadows of diseased gall bladders were found by cholecystography, not to change in size when the pathological change in the wall were moderately advanced." Recently Murphy (87) and Murphy and Higgins (88) have found in the dog that the acutely inflamed gall bladder, induced chemically, will not empty iodized oil after a fat meal. From four to six weeks later, however, the ability of the gall bladder to contract and evacuate returns. They report that lesions were not found outside of the gall bladder. Obviously in the acute condition the various extrinsic forces alleged to cause gall-bladder evacuation were operating but failed to produce an effect. Hence, according to these observations, the acutely inflamed gall bladder does not empty because its contractile mechanism can not be excited to contract.

Does the gall bladder evacuate completely every day? With the evidence at hand, I am convinced that the gall bladder of most normal dogs and cats will evacuate or renew its contents every day on a diet containing adequate fat and protein. On the basis of the cholecystographic observations of Boyden, Whitaker and others, the same statement is true for normal human subjects. Case (41) states that within three hours following a fat meal (two egg yolks, a glass of cream and milk, half and half), the area of the vesicular shadow should be reduced seven-eights or more in a normal subject. Whether or not the gall bladder will renew its contents daily depends on the nature of the diet, the eating habits, and the individual variation. I suspect that if a large series of "normal" subjects were studied, rather wide physiological variations would be found as is true for the colon.

Summary. The gall bladder of all animals studied, including man, possesses contractility and evacuates via the cystic duct, especially on the ingestion of fatty foods. In the dog a maximal gall-bladder contraction exerts a force of from 20 to 30 cm. of bile pressure on its contents. Contraction of the gall bladder is due primarily to hormone stimulation and in part to nervous reflex excitation. The hormone is secretin-like chemically and is produced chiefly by the upper intestinal mucosa upon contact with dilute hydrochloric acid, and very probably certain fatty and other substances which cause secretin production, but which have not yet been tested by cross-circulation experiments. The intravenous injection of the hormone tentatively called cholecystokinin, causes gall-bladder evacuation in animals, including man. The gall bladder empties primarily by the contraction of its musculature. Other factors, such as the "ebb and flow of bile," "elastic recoil," and "intra-abdominal pressure changes," play a minor rôle. The common-duct sphincter and duodenal musculature assist evacuation chiefly by relaxing and thereby permitting bile to pass from the contracting vesicle into the duodenum. The fact that an abnormally tonic common-duct sphincter or duodenum may cause sufficient resistance to overpower the contractile force of the gall bladder and the secretory pressure of the liver is, I believe, of considerable pathologic importance.

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THE ADRENAL CORTICAL HORMONE[1,2]

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[1] In earlier papers the writers expressed the preference for the term "cortical hormone" to designate that principle (or group of principles) present in the adrenal cortex which is necessary for the maintenance of life, and which was shown to be present in significant amount in extracts prepared with lipid solvents. This term was adopted *sub judice* pending definite knowledge of the chemical nature or physiological function of the hormone or hormones involved. Within the past year the yield of hormone per unit weight of adrenal tissue has been increased many times, as has also the physiological potency per unit weight of active fraction. The hormone has not, however, been isolated in a pure crystalline form nor do we as yet know the true function of the adrenal cortex in the animal economy. Consequently it appears to the writers that the present status of the problem does not warrant any change in nomenclature.

[2] These studies were aided by grants from the Josiah Macy, Jr., Foundation of New York.

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1. INTRODUCTION

The adrenal glands were first described in 1563 by Eustachius, but up until the middle of the 19th century they were chiefly objects of curiosity to anatomists, and excited little interest among members of the medical profession. However, in 1855, following the publication by Thomas Addison of the classical paper describing the disease which now bears his name, these glandular structures came into prominence. Since this time they have played an important, if somewhat doubtful rôle, in the speculations and theories of experimental biologists and clinicians.

The discovery of adrenalin and study of its interesting pharmacodynamic properties in the early years of the present century, greatly stimulated experimental and clinical work on the adrenals, and as a result, careful investigation soon established the fact that it is the cortex of the gland that is essential for life, and not the medulla or adrenalin secreting portion. Once this point had been firmly established, the problem of the functional significance of the cortex and the rôle it plays in the economy of the organism presented itself for solution. Although the problem of function is not yet solved, numerous investigators in various laboratories have made significant additions to our knowledge during the past twenty years.

a. Acknowledgment to collaborators

We are not unmindful of the contributions of others to the present subject, but in the space allotted for this paper no attempt will be made to discuss them at this time. We refer especially to the publications of the late Professor Stewart and J. M. Rogoff, Hartman and collaborators, Houssay and collaborators, Britton, Koehler, and Goldzieher. The extensive literature dealing with adrenal anatomy, physiology and chemistry has been adequately covered in several recent reviews. Britton, in a recent review, has summarized the literature up to 1930. Hence this paper is in no sense to be regarded as a review of the adrenal cortex problem, but is merely a summary of the results of a collaborative study of the cortex by the writers and their co-workers, Doctors H. M. Vars and P. A. Bott, both at Princeton, together with Doctors Leonard Rowntree and Carl Greene of the Mayo Clinic, Doctors George Harrop and A. Weinstein of the Medical Clinic of the Johns Hopkins Hospital, and Doctor Bruce Webster of the Department of Medicine, College of Physicians and Surgeons, Columbia University.

b. General statement of the problem

The function of the adrenal cortex is unknown at the present time. True, various theories have been advanced but none of them are based upon exact, well controlled, experimentation, and they merit little consideration. For the most part, those who have worked most extensively in the field of adrenal physiology, have frankly stated their ignorance of the rôle that the cortex plays in the organism. After some years of experimentation upon adrenal cortical function, the writers became convinced that the best method of attack upon the problem was to endeavor to obtain an active preparation of the adrenal cortical hormone and by means of it, to study adrenalectomized animals, subjected to various dosage over long periods. The fact that our knowledge of the cortex is so meager has rendered the problem of isolating an active extract from the gland exceedingly difficult.

2. METHOD OF ASSAY USED IN DEVELOPING A PHYSIOLOGICALLY POTENT EXTRACT OF THE CORTEX

In our early work we found, as a consequence of the confused state of present day knowledge of cortical function, that the only reliable criterion for testing the activity of an extract was its effect upon the life-span of bilaterally adrenalectomized animals. If an extract failed to keep a series of adrenalectomized animals alive and in normal health, at least for a considerable period over and beyond the maximum limit of the life-span of control non-treated adrenalectomized animals, it was regarded as inactive.

TABLE 1

*Survival period of adrenalectomized cats**

SERIES	SEX	INTERVAL BETWEEN OPERATIONS	SURVIVED AFTER SECOND OPERATION
		days	*days*
E. 1	Male	23	5
E. 2	Female	23	4
E. 3	Female	25	8
E. 4	Female	25	5
E. 6	Male	24	7
F. 6	Male	31	5
F. 7	Female	26	5
F. 9	Female	28	13
F. 10	Male	26	5
F. 16	Male	13	11
F. 20	Male	12	8
H. 1	Male	10	8
H. 2	Female	10	7
H. 3	Female	10	6
H. 4	Female	10	6
H. 5	Male	8	9
H. 6	Male	7	5
H. 7	Female	7	8
H. 8	Female	8	9
H. 9	Female	8	4
H. 10	Male	7	4
H. 15	Male	12	8
H. 16	Male	32	15
H. 17	Male	28	14
H. 18	Male	22	8
H. 19	Male	36	8
H. 20	Male	7	5
H. 21	Male	12	7
H. 24	Male	10	8
H. 27	Male	8	6
H. 30	Male	9	9
H. 31	Male	9	7
H. 32	Female	7	14
H. 33	Male	7	7
H. 34	Male	7	7
H. 35	Male	8	15
H. 36	Male	7	6
H. 37	Female	15	10
N. 1	Male	18	11
N. 2	Male	7	11
N. 3	Male	16	9
N. 4	Male	7	6

* Adult animals.

TABLE 1—*Continued*

SERIES	SEX	INTERVAL BETWEEN OPERATIONS	SURVIVED AFTER SECOND OPERATION
		days	*days*
N. 5	Female	7	14
N. 6	Male	8	15
N. 7	Male	7	6
N. 8	Male	7	6
N. 9	Female	11	9
N. 10	Male	8	8
N. 11	Male	7	8
N. 12	Male	7	8
N. 13	Female	8	9
N. 14	Male	8	7
N. 15	Male	8	7
N. 16	Male	10	11
N. 17	Male	10	7
N. 18	Female	8	8
N. 19	Male	9	6
N. 20	Female	8	6
N. 21	Male	7	12
N. 22	Male	7	15
N. 23	Female	8	10
N. 24	Male	9	7
N. 25	Male	9	10
N. 26	Male	20	14
N. 27	Female	8	5
N. 28	Male	8	6
N. 29	Female	7	6
N. 30	Male	3	10
N. 32	Female	13	6
N. 33	Male	22	18
N. 34	Male	16	6
N. 35	Male	4	5
N. 36	Female	5	7
N. 37	Male	4	5
N. 38	Female	7	9
N. 39	Male	5	5
N. 40	Male	7	7
N. 41	Male	7	8
N. 42	Male	10	13
N. 43	Male	7	9
N. 45	Male	16	9
K. 1	Male	9	11
K. 2	Male	9	25
K. 3	Male	11	13
K. 4	Male	11	11
K. 5	Male	5	10

TABLE 1—*Concluded*

SERIES	SEX	INTERVAL BETWEEN OPERATIONS	SURVIVED AFTER SECOND OPERATION
		days	*days*
C. 1	Male	6	7
C. 2	Male	13	8
C. 3	Male	13	7
C. 4	Female	12	9
C. 5	Male	6	9
C. 6	Male	7	10
C. 7	Female	4	11
C. 8	Male	17	5
C. 9	Male	6	12
C. 10	Male	8	16
P. 1	Male	7	7
P. 2	Male	7	11
P. 3	Male	6	7
P. 4	Male	5	6

The first problem confronting us at that time, was to determine the average life-span of a large number of control adrenalectomized cats. In a control series of 38 animals, the average life span for the group was 7.7 days. Recently our control series has been extended to 100 cats, with an average life-span of 8.6 days. Only rarely have we encountered accessory glands in the several hundred animals studied. In our experience with over 300 adrenalectomized animals about three per cent had them. All animals were autopsied after completion of the experiment, and careful search made for accessary cortical glands.

3. PREPARATION OF ADRENAL CORTICAL EXTRACT

In reviewing the experimental work of this laboratory on the development of a physiologically potent extract of the adrenal cortex suitable for clinical use, it is important to bear in mind two of the major obstacles confronting us in the early part of this investigation. One of these was the sensitivity of the test object, the adrenalectomized cat, to extraneous influences, and the other was the presence in the adrenal gland of 0.3 to 0.4 per cent of adrenalin. It had been known for a long time that adrenalectomized animals are very sensitive to practically anything which throws a strain on the physiological balance of the organism such as excessive heat or cold, bacterial toxins, certain organic basis and alkaloids, etc. Because of this derogatory influence of extraneous factors on the test animals, we were always confronted with the possibility of a toxic action of a crude extract masking the presence of appreciable quantities of cortical hormone. This possibility was enhanced by the probable contamination of crude extracts with appreciable quantities of adrenalin. It is our impression that adrenalectomized animals suffering from adrenal insufficiency are hyper-sensitive to pharmacological doses of adrenalin. Consequently, the problem consisted of finding a method for the extraction of the cortical hormone, which would at the same time separate the cortical hormone from adrenalin. This set of circumstances is clearly illustrated by the fact that the crude extract obtained by extracting adrenal tissue with alcohol is very highly toxic, not only to adrenalectomized cats but also to normal animals. Nevertheless on further purification comparable doses readily relieve the syndrome of adrenal insufficiency in adrenalectomized cats and dogs.

In developing a fractionation technique, the interesting observation was made that the presence of lipid material, particularly phospholipid, markedly influences the solubility of adrenalin in lipid solvents. For example 50 cc. of benzene in the presence of solid adrenalin does not dissolve sufficient adrenalin to give a color reaction with the Folin, Cannon and Denis reagents. However, 50 cc. of a 2.5 per cent solution of lecithin in benzene will readily dissolve 100 mgm. of adrenalin. Because of this change in solvent properties, the earlier lipid fractions contained appreciable quantities of adrenalin from a physiological standpoint. In order to keep the troublesome question of adrenalin at a minimum in the early work, while adequate methods of fractionation were being developed, we resorted to the dissection of the adrenal glands. It was thought that the difficulty attendant on the presence of adrenalin might be completely surmounted by dissection of the glands at the abattoir immediately after removal from the animals. The data presented in table 2 show quite clearly that no advantage was gained by such a procedure, since cortical tissue dissected at the abattoir contains almost as much adrenalin as cortical tissue obtained from glands which were dissected 20 hours after removal from the animal. The glands used in the above analyses were handled very carefully so as to keep diffusion processes at a minimum. In routine work in the laboratory it is impractical to dissect very carefully large numbers of adrenal glands. Consequently the tissue used in the preparation of our earlier extracts contained more adrenalin than the data in table 2 would indicate. Freshly dissected ground cortical tissue in routine work assayed about 2 to 2.5 mgm. of adrenalin per gram of tissue (colorimetric).

In 1929 the writers obtained and reported clear cut evidence that the cortical hormone could be readily extracted with organic lipid solvents. It was also established that the hormone was relatively stable in both ethyl alcohol and benzene, and these two solvents were used therefore as a basis for fractionation procedures designed to yield an extract sufficiently active and pure for clinical use in Addison's disease. The lipid fraction obtained by the alcohol-benzene process is further purified by treating with acetone. The acetone-insoluble material consisting chiefly of phospholipid is inert. The acetone-soluble fraction is further purified by selective distribution between 70 per cent alcohol and petroleum-ether. The petroleum-ether-soluble fraction is inactive. The 70 per cent alcohol-soluble fraction contains the cortical hormone along with some adrenalin. Our earlier aqueous extracts were made directly from the 70 per cent alcohol-soluble fraction.

TABLE 2

Adrenalin content of adrenal glands (beef)

	MILLIGRAMS ADRENALIN PER GRAM TISSUE (BIO-ASSAY)
Cortex dissected from medulla while glands still warm (about 20 minutes after death of animal):	
Cortex	0.7
Medulla	10.0
Whole glands stored in refrigerator (8°C.) for 20 hours before dissection:	
Cortex	1.0
Medulla	12.0

4. EFFECT UPON THE LIFE-SPAN OF ADRENALECTOMIZED CATS

The results obtained by administration of this type of extract to adrenalectomized cats were so striking and consistent, that three animals were sacrified on the 50th day of survival in order to satisfy our-

TABLE 3

Survival period of adrenalectomized cats treated with the alcohol-soluble fraction

SERIES	SEX	INTERVAL BETWEEN OPERATIONS	SURVIVAL AFTER 2ND OPERATION	REMARKS
		days	*days*	
M-1	Female	7	50	1.5 cc. twice daily. Weight 2555 grams. Animal sacrificed on 50th day for autopsy. Accessory tissue not found
M-2	Female	6	50	1 cc. twice daily. Weight 1675 grams. Sacrificed on 50th day. No accessories present
M-3	Male	8	50	1.5 cc. twice daily. Weight 2725 grams. Sacrificed on 50th day. No accessories present
M-4	Male	8	47	1 cc. twice daily. Weight 2290 grams. Died pneumonia. No symptoms adrenal insufficiency noted
M-5	Male	8	109	1.5 cc. twice daily. Weight 3185 grams. Extract discontinued on 100th day
M-6	Female	12	113	1.5 cc. twice daily. Weight 2270 grams. Extract discontinued on 100th day
M-7	Male	18	112	1.5 cc. twice daily. Weight 2810 grams. Treatment discontinued on 100th day
M-8	Female	6	163	1.5 cc. twice daily. Weight 2700 grams. Extract discontinued on 100th day
M-9	Male	7	107	1.5 cc. twice daily. Weight 3200 grams, when treatment began. Extract discontinued on 100th day
M-10	Male	7	115	1.5 cc. twice daily. Weight 2835 grams. Extract discontinued on 100th day
M-11	Male	6	125	1.5 cc. twice daily. Weight 2905 grams. Extract discontinued on 100th day
M-12	Male	7	56	1 cc. twice daily. Weight 2180 grams. Cause of death unknown. Never presented symptoms
M-13	Male	10	111	1.5 cc. twice daily. Weight 2720 grams. Extract discontinued on 100th day
M-14	Male	8	118	1.5 cc. twice daily. Weight 3102 grams. Extract discontinued on 100th day
M-15	Male	10	58	1.5 cc. twice daily. Weight 3330 grams. Developed severe generalized infection. Killed. No accessory glands present
M-16	Male	10	117	1.5 cc. twice daily. Weight 2630 grams. Extract discontinued on 100th day
M-17	Female	9	56	1.5 cc. twice daily. Weight 3420 grams. Developed severe infection. Killed. No accessory tissue present
M-18	Male	10	117	1.5 cc. twice daily. Weight 3730 grams. Extract discontinued on 100th day
M-19	Male	8	120	1.5 cc. twice daily. Weight 3215 grams. Extract discontinued on 100th day

selves that no question of accessory cortical glands was involved. No trace of accessory tissue was found, so that the remainder of the series which were on hand at the time were continued on the treatment. Since all of the cats survived in excellent condition and gained weight rapidly, it became obvious that they would probably survive indefinitely so long as the treatment continued. As our laboratory facilities for keeping animals were extremely limited, it became necessary to fix an arbitrary period of survival at the end of which the extract treatment would be discontinued, and the animals permitted to die. A survival period of 100 days was selected as the test period, and the administration of extract stopped at the end of this interval. Data on adrenalectomized cats treated with the alcohol-soluble fraction are recorded in table 3.

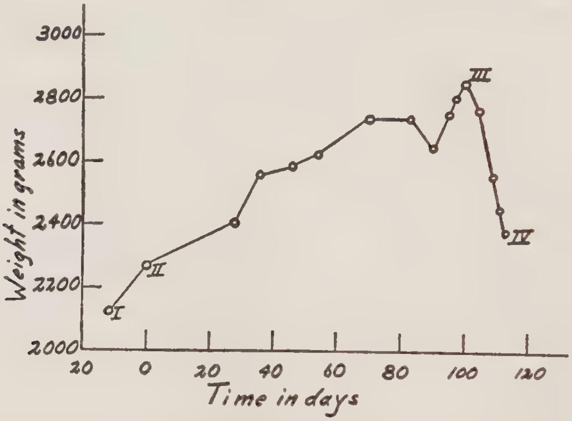

Fig. 1. Weight Chart of a Bilaterally Adrenalectomized Cat Treated with Cortical Hormone

I, Right adrenal removed; II, Left adrenal removed, treatment started 24 hours later; III, treatment discontinued; IV, death from adrenal insufficiency.

In evaluating the experiment, it should be borne in mind that the animals were in perfect health at the time of cessation of extract treatment, and had gained steadily in weight. After withdrawal of treatment the animals died with typical symptoms of adrenal insufficiency within a short time with one exception. The exceptional case survived 63 days and lost over one third of its weight and presented symptoms of chronic insufficiency, throughout the period.

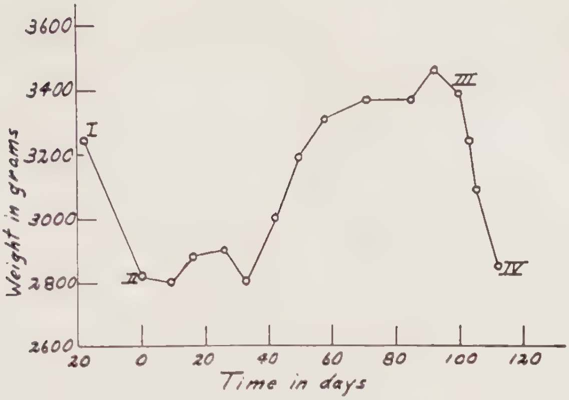

Fig. 2. Weight Chart of a Bilaterally Adrenalectomized Cat Treated with Cortical Hormone

I, Right adrenal removed; II, Left adrenal removed, treatment started 24 hours later; III, treatment discontinued; IV, death from adrenal insufficiency.

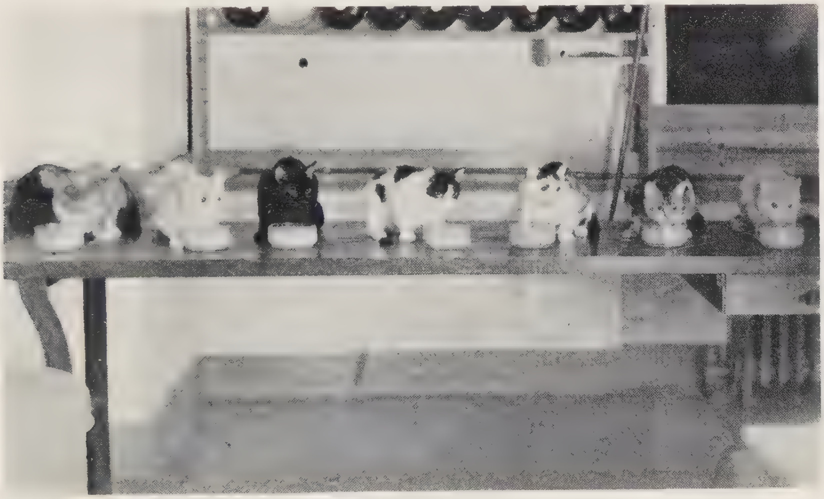

Fig. 3. A Series of Seven Bilaterally Adrenalectomized Cats Treated with Cortical Hormone

The picture was taken by flashlight 100 days after the removal of the adrenal glands. All seven animals died with typical symptoms of adrenal insufficiency within three weeks after withdrawal of treatment.

The behavior of the long surviving cats was perfectly normal so far as we were able to judge. They ate heartily, played about, fought with one another, and in two instances males were observed to attempt copulation with females newly introduced into the laboratory. Persons viewing the animals, and without knowledge of their history, were unable to distinguish the adrenalectomized extract-treated cats from normal unoperated animals. The pertinent data on the first animal treated with the alcohol-soluble fraction are given in table 4.

TABLE 4

No. 1. Male

DATE	WEIGHT	REMARKS
	grams	
December 16, 1929	3,160	Right adrenal removed
December 24, 1929	3,185	Left adrenal removed. Treatment started December 25
January 28, 1930	3,320	
February 12, 1930	3,300	
March 5, 1930	3,420	
March 12, 1930	3,500	
March 22, 1930	3,765	
March 30, 1930	3,820	
April 3, 1930	3,835	Extract discontinued. Animal in perfect health
April 7, 1930	3,630	
April 8, 1930	3,610	Symptoms of suprarenal insufficiency
April 9, 1930	3,515	Symptoms of suprarenal insufficiency
April 10, 1930	3,400	Marked symptoms
April 11, 1930	3,355	Prostration, death

In figures 1 and 2 are shown the weight charts of two typical cases of adrenalectomized cats treated with the alcohol-soluble fraction for 100 days. Following withdrawal of extract the animals promptly died presenting the typical symptoms of adrenal insufficiency. The marked weight increases shown by these animals are considered as merely an indication of good physiological and nutritive condition and are probably not due to any specific influence of the cortical hormone per se.

5. REMOVAL OF LAST TRACES OF ADRENALIN FROM THE EXTRACT

The type of extract employed in the experiments concerned with long survival contained appreciable quantities of adrenalin, 0.17 to

0.37 mgm. per cubic centimeter. When used in small doses such as 0.5 to 1 cc. per kilogram of body weight subcutaneously, it gave excellent results, and the cats remained normal. Extract containing this amount of adrenalin is unsuitable for intravenous or intraperitoneal injection since a small quantity gives rise to symptoms of adrenalin over-dosage.

We were eager to test the extract upon untreated cats prostrate and on the verge of death from adrenal insufficiency, but owing to the adrenalin present, were unable to use the early aqueous extracts on cats presenting such severe symptoms. It became necessary, therefore, to devise a method for the removal of practically all of the adrenalin, without diminishing thereby the potency of the extract. Consequently further fractionation studies were undertaken with the primary object of separating adrenalin from the cortical hormone. A very simple, but effective method of fractionation was devised which in our hands has given very consistent results, both from the standpoint of yield and purity, and the cortical extract obtained by this method is suitable for subcutaneous, intraperitoneal or intravenous use.

The fractionation step is based upon the observation of Whitehorn (1923) that adrenalin is removed quantitatively from solution, by passing the solution through permutit.[3] In a comprehensive study of the use of permutit as a reagent for the separation of amines from other nitrogenous substances Whitehorn found that in general the bases removed were those having a basic dissociation constant of 5×10^{-9} or greater. He also observed that permutit takes up amines from organic solvents as well as from aqueous solution. Permutit has the unique property of taking up bases by means of its base-trading reaction. It is not a simple adsorption reaction. The sodium of permutit is replaced by the weak base which can then later be liberated by the addition of an excess of sodium ion. This is best illustrated by the reversible reaction.

$$NH_4Cl + \text{Na permutit} \rightleftharpoons NH_4 \text{ permutit} + NaCl$$

We have found permutit to be a very valuable reagent for removing adrenalin and much inert material, including most of the pigment from the active adrenal cortex fraction.

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[3] Permutit is a synthetic zeolite used industrially for softening hard water and refining sugar. It was used in the determination of urinary ammonia by Folin and Bell.

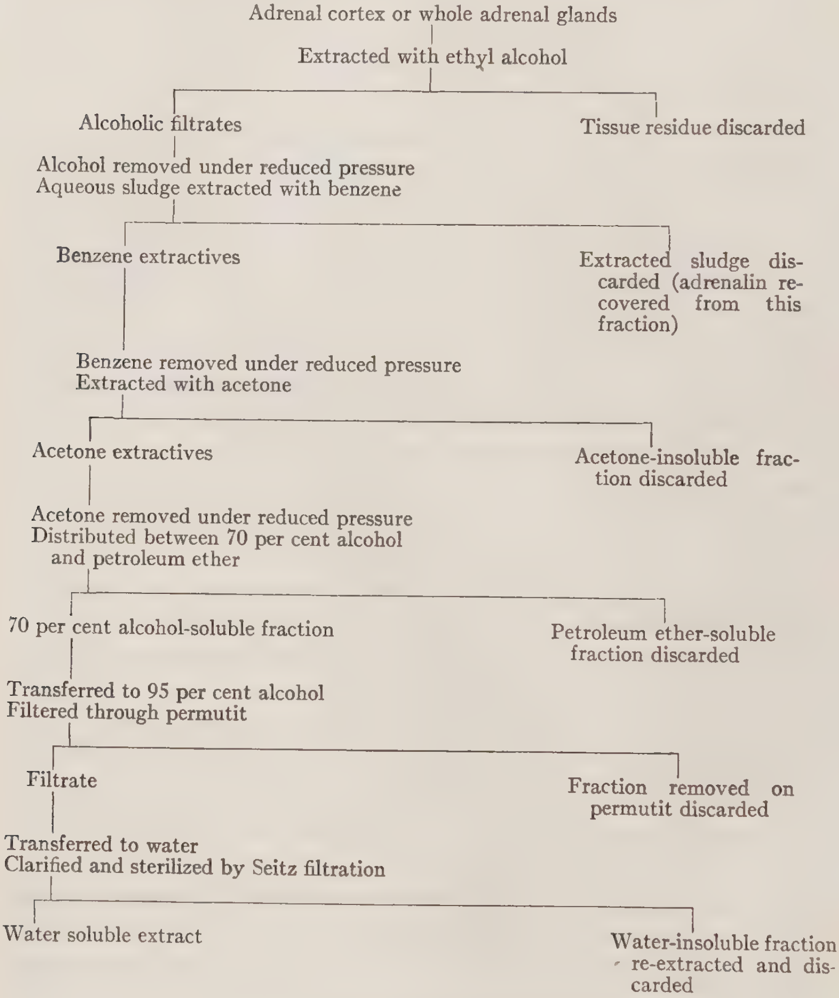
Adrenal cortex or whole adrenal glands
Extracted with ethyl alcohol
Alcoholic filtrates
Tissue residue discarded
Alcohol removed under reduced pressure
Aqueous sludge extracted with benzene
Benzene extractives
Extracted sludge discarded (adrenalin recovered from this fraction)
Benzene removed under reduced pressure
Extracted with acetone
Acetone extractives
Acetone-insoluble fraction discarded
Acetone removed under reduced pressure
Distributed between 70 per cent alcohol and petroleum ether
70 per cent alcohol-soluble fraction
Petroleum ether-soluble fraction discarded
Transferred to 95 per cent alcohol
Filtered through permutit
Filtrate
Fraction removed on permutit discarded
Transferred to water
Clarified and sterilized by Seitz filtration
Water soluble extract
Water-insoluble fraction re-extracted and discarded

Fig. 4. Method of Fractionation for the Preparation of Adrenal Cortical Hormone

The 70 per cent alcohol-soluble fraction containing the cortical hormone serves as the starting point. The solvents are removed by distillation under reduced pressure and the residue taken up in 95 per cent alcohol. The solution is then filtered through permutit. The permutit is washed with 95 per cent ethyl alcohol after the alcoholic solution of the active fraction has entered the filter. Following concentration the filtration procedure is repeated using smaller portions of permutit. The alcoholic solution is then concentrated and distilled water is added. The amount of water added at this point depends upon the concentration of extract desired. The alcohol is removed and the extract diluted to volume with distilled water. The material

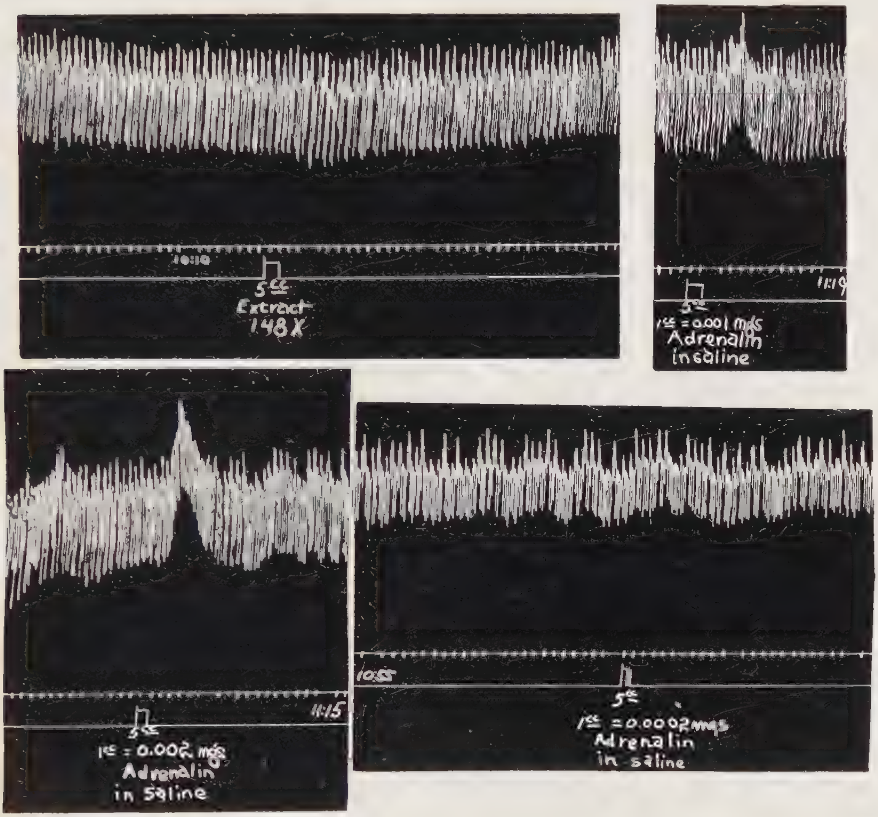

Fig. 5. Effect of Cortical Extract upon Blood Pressure

Dog. Male. Weight 8 kgm. Morphine-ether anesthesia. Nonatropinized and with vagi intact. Time interval 5 seconds. One cubic centimeter of extract employed equivalent to 30 grams of adrenal cortex. Five cubic centimeters of solution injected in all cases.

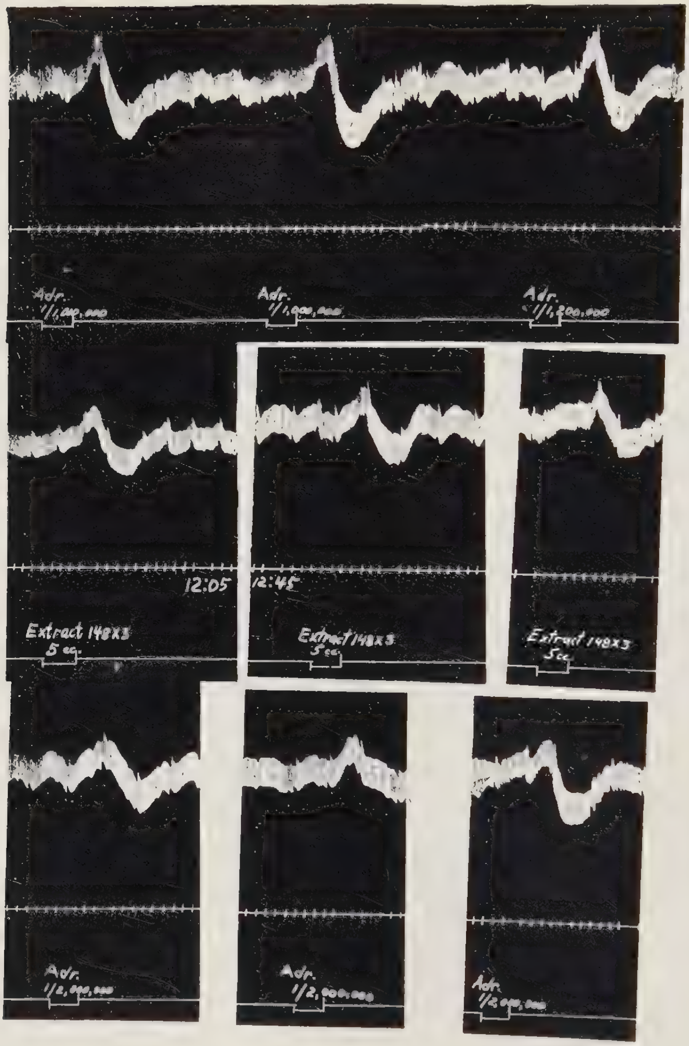

Fig. 6. Effect of Cortical Extract upon Blood Pressure

Dog. Male. Weight 10 kgm. Morphine-ether anesthesia. Atropinized and with vagi cut. Time interval 5 seconds. One cubic centimeter of extract employed equivalent to 30 grams adrenal cortex. Five cubic centimeters of solution injected in all cases. Adrenalin content approximately 1:2,000,000.

is then clarified and sterilized by passing through a Seitz filter. The filtered extract is crystal clear and very pale yellow in color. It contains 0.2 to 0.3 gram of solids per 100 cc. including approximately 0.05 mgm. of adrenalin. The pH (quinhydrone) is between 5 and 6. The addition of 0.8 per cent sodium chloride, renders the extract isotonic. One cubic centimeter represents 30 grams of fresh beef adrenal cortex. The flow sheet of the fractionation procedure is given in figure 4.

The effect of permutit fractionation in removing adrenalin from the extract, is well shown in the experiments upon blood pressure. According to our bio-assay, using blood pressure of dogs, a single filtration of the extract through permutit (20 grams of permutit per kilogram of cortex) reduces the adrenalin content to less than 0.01 mgm. of adrenalin per cubic centimeter. Material purified by an additional filtration through permutit (10 grams per kilogram of cortex) contains but a minute amount of adrenalin—between 1:1,500,000 and 1:2,000,000. We now have preparations assaying around 1:5,000,000 adrenalin. It is obvious that permutit practically quantitatively removes adrenalin from the cortical hormone. Figures 5 and 6 show typical assay data (blood pressure) on the adrenalin content of this type of extract.

6. REVIVAL OF COMATOSE ADRENALECTOMIZED CATS

The potency of the hormone produced by permutit fractionation was tested upon bilaterally adrenalectomized cats presenting very severe symptoms of adrenal insufficiency. The animals were prostrate and unable to walk, and if placed upon their feet, promptly collapsed. The body was cold and clammy to touch, and the rectal temperature had fallen from the normal of 101.5° to 94° or below. Death was imminent in all cases. Subcutaneous, intraperitoneal, or intravenous injections of the extract into comatose animals restores them to normal condition without symptoms within 48 to 72 hours following the first injection. When the intravenous method is employed the animals may return to normal health within 24 hours. The effect of the cortical hormone upon adrenalectomized animals verging on death from adrenal insufficiency is amazing. Moreover, such animals have been restored to health within three days, and then kept normal for months, until we voluntarily terminated the experiment by withdrawing extract treatment. It is quite simple to bring the animal repeatedly into severe insufficiency by withholding extract, and then to return the animal to normal by again giving treatment. Figure 7 shows the weight chart of an adrenalectomized cat permitted to develop very severe symptoms of adrenal insufficiency. The animal was then returned to normal with the cortical hormone, and kept for 100 days on small daily doses.

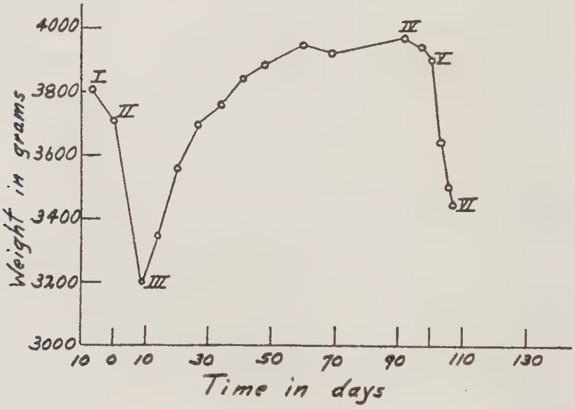

Fig. 7. Weight Chart of Bilaterally Adrenalectomized Cat Treated with Cortical Hormone, Following the Exhibition of Severe Symptoms of Adrenal Insufficiency

I, right adrenal removed; II, left adrenal removed; III, animal prostrate; treatment begun; IV, dosage reduced to 0.25 cc. per kilogram daily; V, treatment discontinued; VI, death from adrenal insufficiency.

7. USE OF WHOLE ADRENAL GLANDS IN PREPARATION OF EXTRACT

Fractionation with permutit has made possible the preparation of active extracts from whole beef adrenal glands thereby doing away with the expense of dissection. Potent extracts were prepared by simply extracting whole adrenal glands (freed from extraneous fat and connective tissue) by the process previously described for preparation of extracts from dissected cortex. Each cubic centimeter of whole gland extract represents 40 grams of whole adrenal glands. The adrenalin concentration of the finished extracts varies between 1:1,250,000 and 1:2,500,000 while the solid content ranges from 0.2

to 0.3 per cent. The following two sample protocols taken from the many animals studied in this connection, show quite clearly that extracts made from whole adrenal glands are active and will restore to apparently normal condition, cats prostrate from adrenal insufficiency. Subcutaneous administration is just as effective as intraperitoneal use. Whole gland extract can also be used intravenously. An abbreviated protocol of one of our typical cases is included here.

Protocol W. G. 1. Adult male. Weight 2700 grams. Right gland removed September 1, 1930. Left gland removed September 6. Weight 2700 grams. September 6–11, no symptoms. September 11, first symptoms noted. September 12, 8 p.m., cat exhibited severe symptoms. Rectal temperature 94°, animal staggers about when placed upon its feet, given 7 cc. extract intraperitoneally at 9 p.m. Weight 2550 grams. September 13, animal about the same as previous day. Rectal temperature 96°. Given two injections of 7 cc. each. September 14, cat bright and lively, eats liver eagerly, walks about in a normal manner. Rectal temperature 100.2°. Given two 7 cc. injections during day. September 15, cat eats liver and drinks milk. Rectal temperature 101.6°, no symptoms. Extract discontinued. Weight 2746 grams. September 16–19, cat normal. Sep-

FIG. 8. BILATERALLY ADRENALECTOMIZED FEMALE AND MALE DOGS ABOUT ONE YEAR FOLLOWING OPERATION

Both animals were repeatedly brought into insufficiency by withdrawal of extract tember 19, symptoms again appeared. September 20, cat prostrate. Animal died at 8 p.m. Weight 2541 grams.

Within the past year we have confined our attention chiefly to adrenalectomized dogs. Such animals respond to treatment similarly to cats, and we have in our laboratory at the present moment, animals adrenalectomized for periods ranging from 5 to 12 months, which have been repeatedly thrown into adrenal insufficiency and repeatedly restored to normal. Figure 8 shows two adrenalectomized dogs about one year following adrenalectomy. Both animals were repeatedly thrown into insufficiency by the gradual or total withdrawal of extract.

It is quite evident that permutit fractionation not only separates adrenalin from the cortical hormone, but leaves the potency of the latter unimpaired. Reference will be made later to the use of this type of extract in the clinic in the treatment of Addison's disease.

8. QUANTITATIVE METHOD OF ASSAY—THE DOG UNIT

In developing a technique for the biological assay of the cortical hormone, there are obviously three possibilities: (1) assay based on the effect of the hormone on the normal animal, (2) assay based on the minimum amount of hormone necessary to bring an adrenalectomized animal from a condition of insufficiency to a normal state, (3) assay based on the minimum amount of hormone necessary to maintain normal physiological condition in the adrenalectomized animal.

Doctors Harrop and Weinstein have devoted a great deal of time and effort in devising a suitable assay method. After months of experimentation they chose the change in blood non-protein nitrogen and urea of the bilaterally adrenalectomized dog as offering the simplest and easiest method to quantitate. The cortical hormone was supplied from the Princeton Laboratory and the assay data collected at the Johns Hopkins Hospital. The following discussion of the method of bio-assay and the dog unit is based upon their data, a preliminary report of which was published in collaboration with the writers.

Since extensive experimentation has failed to reveal a reaction of the normal animal to cortical hormone administration, it has become increasingly clear that for the time being, adrenalectomized animals must be used for standardization purposes. There is no known criterion for determining accurately the degree of insufficiency from which an adrenalectomized animal is suffering, and consequently standardization cannot be based on the return of an animal suffering from adrenal insufficiency to a normal physiological condition. We have, therefore, accepted the other alternative, that is, the maintenance of normal physiological condition in the adrenalectomized animal. During recent metabolic studies on bilaterally adrenalectomized dogs, permitted to go into adrenal insufficiency by the withdrawal of daily injections of cortical hormone, it was noted that the earliest indication of a deviation from normality is a rise in the blood-protein and in the urea nitrogen. We have utilized this observation as a basis for biological assay of the extracts.

Adult male dogs, about 6 to 10 kgm. body weight are selected, free of infection and worm infestation. They are adrenalectomized in 2 stages, and the success of the operation is established after complete healing of the wounds by production of insufficiency symptoms by withdrawal of extract. The dogs are then placed in separate cages and given 0.5 cc. extract per kilogram in 2 equally divided doses daily, morning and evening, subcutaneously, until it is demonstrated that the blood non-protein-nitrogen (or urea) is at a normal and constant level, that the weight is maintained, the food properly eaten, and the clinical condition in every respect normal. The ration given consists of 80 calories per kilogram per day of Bal-Ra dog ration, with 1 cc. cod liver oil three times weekly, and 1 gram vitavose per kilogram three times weekly on alternate days. The dogs are permitted to run in open air, weather permitting, at least two hours daily. When normal constant conditions are assured the per kilogram dosage is progressively reduced at 7 to 10 day intervals, usually from 0.5 cc. per kilogram to 0.25 cc., then to 0.13 cc. and so on, until the blood non-protein-nitrogen shows a definite rise (15 mgm. or to a level above 50 mgm. per 100 cc.) followed by anorexia and the other clinical evidences of imminent adrenal insufficiency. Dissected cortex extract (1 cc. represents 30 grams beef adrenal cortex) prepared by methods previously described will maintain the animals in normal condition at a level of from 0.25 cc. to 0.1 cc. per kilogram, the minimum dose varying with different batches of extract. At the present time we are using 2 animals for each assay.

A dog unit (D.U.) is defined as the minimum daily kilogram dose of cortical hormone necessary to maintain normal physiological conditions in the adrenalectomized dog for a period of 7 to 10 days; the two criteria of normal physiological condition being maintenance of body weight and blood level of non-protein nitrogen (or urea). According to this definition the hormone content of different batches of dissected cortex extract varies from 4 to 10 dog units per cubic centimeter.

Typical assay data are given in table 5. Data on non-protein nitrogen content of the blood are not included since in our experience the non-protein nitrogen and urea curves run parallel.

TABLE 5

Assay of dissected cortex extract

Extract No. 813A: 1 cc. = 30 grams cortex; 1 cc. = 2.2 mgm. solids.

Dog 7

WEIGHT	DAILY DOSE PER KILOGRAM			DAYS	UREA N	CLINICAL CONDITION
	Volume	Solids	Cortex			
kgm.	*cc.*	*mgm.*	*grams*		*mgm. per 100 cc.*	
7.5	0.25	0.55	7.5	7	18–23	Normal
7.9	0.2	0.44	6.0	7	23–25	
7.8	0.13	0.29	3.9	8	25–26	
7.6	*0.1*	*0.22*	*3.0*	7	26–33	Appetite failing
6.7	0.076	0.17	2.3	2	33–59	Definite insufficiency

Yield per kilogram cortex = 333 D.U.

Relative to the technique of assay which we are using at the present time, it should be emphasized that the level of blood non-protein nitrogen or urea is taken as merely indicative of the general clinical condition of the animal. It is pointed out elsewhere that a marked rise in urea is accompanied or soon followed by varying degrees of anorexia, asthenia, or at least general lassitude, as well as loss in body weight. However, the level in blood urea is the simplest objective finding which can be readily followed and expressed quantitatively. On the other hand, the degree of elevation of blood urea cannot be strictly correlated with the clinical condition. Some animals succumb with an elevation of one hundred per cent while others die with an elevation of several hundred per cent. However, all adrenalectomized dogs thus far observed dying of adrenal insufficiency show a marked elevation of blood urea.

9. FURTHER STUDIES OF EXTRACTION

a. Comparison of yield of hormone from dissected cortex and whole gland

With the activity of dissected cortex extract established at 4 to 10 D. U. per cubic centimeter (the potency varying with different batches) it appeared to be of interest to determine the relative potency of whole gland extract since earlier qualitative work showed that dissection was unnecessary. Much to our surprise we found that extracts prepared from whole glands are many times as potent as those prepared from dissected cortex (on an equivalent weight basis). Whole gland extract (1 cc. represents 40 grams whole beef adrenal gland) contains 40 to 80 D.U. per cubic centimeter. Table 6 is a summarized comparison of whole gland and dissected cortex extract.

TABLE 6

WHOLE GLAND	DISSECTED CORTEX
1,000 grams	720 grams (average)
57.5 mgm. active fraction	60 mgm. active fraction (average)
25 cc. extract	24 cc. extract
40 to 80 D.U. per cubic centimeter	4 to 10 D.U. per cubic centimeter
Yield 1,000 to 2,000 D.U.	Yield 96 to 240 D.U.

It should be noted that whereas the yield of hormone by whole gland extraction is increased many times, nevertheless the solid content of the extract is about the same as that prepared from dissected cortex. Assay data presented in table 7 show clearly the high yield of hormone obtained by whole gland extraction.

b. Non-effect of freezing the glands for long periods of time upon the yield of hormone

From the standpoint of large scale production of the cortical hormone for laboratory and clinical use, it was of considerable importance to determine the effect on the yield of freezing the glands for long periods of time. In table 8 are presented assay data on an extract prepared from glands which were frozen solid for one month prior to extraction. Comparable data were obtained with an extract prepared from glands which were frozen for 5 months. Freezing has no demonstrable effect on the yield of hormone or the purity of the product obtained.

TABLE 7

*Assay of whole gland extract**

Extract No. 150–P6: 1 cc. = 50 grams gland; 1 cc. = 2.4 mgm. solids.

Dog 12

WEIGHT	DAILY DOSE PER KILOGRAM			DAYS	UREA N	CLINICAL CONDITION
	Volume	Solids	Gland			
kgm.	*cc.*	*mgm.*	*grams*		*mgm. per 100 cc.*	
8.2	0.25	0.6	12.5	7	25–28	Normal
8.2	0.125	0.3	6.25	8	25–29	
8.5	0.1	0.24	5.0	8	27–29	
8.5	0.07	0.31	3.5	9	27–33	
8.2	0.05	0.12	2.5	7	33–33	
8.6	0.025	0.06	1.25	25	28–33	
8.1	0.0	0.0	0.0	5	28–90	Definite insufficiency

* It will be noted that this assay is incomplete since the dosage was not reduced to the minimum for maintenance. When the dog remained normal and free from all symptoms on 0.025 cc. per kilogram per day for 25 days extract was discontinued.

TABLE 8

Assay of whole frozen gland extract

Extract No. 1014F: 1 cc. = 40 grams gland; 1 cc. = 2.4 mgm. solids.

Dog 7

WEIGHT	DAILY DOSE PER KILOGRAM			DAYS	UREA N	CLINICAL CONDITION
	Volume	Solids	Gland			
kgm.	*cc.*	*mgm.*	*grams*		*mgm. per 100 cc.*	
7.6	0.25	0.6	10	7	24–27	Normal
8.2	0.125	0.3	5	7	22–24	Normal
8.2	0.1	0.24	4	7	24–25	Normal
8.2	0.07	0.17	2.8	7	24–29	Normal
8.2	0.05	0.12	2	14	27–30	Normal
7.6	0.031	0.07	1.2	11	27–34	Slightly less active; variable appetite
7.5	0.025	0.06	1.0	16	28–34	
7.5	*0.0125*	*0.03*	*0.5*	14	30–53	
7.2	0.006	0.014	0.24	1	53–70	Definite insufficiency

Yield per kilogram gland = 2,000 D.U.

A very good yield of hormone was obtained from glands which had autolyzed for 48 hours at room temperature. Extracts preserved with 0.1 per cent benzoic acid at 5°C. retained their activity for 6 months. Longer periods have not been studied. The stability of the finished extract has been checked in two ways. (1) Adrenalectomized dogs have been kept on a single batch of extract for periods of 3 to 5 months. On withdrawing extract they have come promptly into insufficiency. (2) Assayed extract has been set aside for 6 months and reassayed without showing any loss in potency.

c. Simplification of technique in preparing the extract

In developing the original method of fractionation we resorted to the precaution of thorough extraction of the respective fractions in order to keep the yield of hormone as high as possible. We have found that whole gland extracts of approximately equal potency, but lower solid content, can be prepared by using the same fractionation procedure as previously described, but decreasing by 50 per cent the thoroughness of extraction of the respective fractions. In this simplified technique the glands are extracted once with alcohol for 48 hours, the benzene-soluble fraction is extracted twice with acetone, the acetonsoluble fraction is distributed twice between 70 per cent alcohol and petroleum-ether, and the alcohol-soluble fraction is filtered only once through permutit. The adrenalin concentration is less than 1:2,000,000 (blood pressure-dog). This constitutes our so-called "half"

TABLE 9

FRACTIONATION STEPS	STANDARD METHOD	HALF METHOD
1. Alcohol extraction	First, $2\frac{1}{2}$ volumes 95 per cent; second, 2 volumes 80 per cent	$2\frac{1}{2}$ volumes 95 per cent for 2 days
2. Benzene extraction	Until last washing pale straw or colorless	Same
3. Acetone extraction	3 extractions	2 extractions
4. Petroleum ether—70 per cent alcohol distribution	4 distributions	2 distributions
5. Permutit filtration	First, 10 grams per kilogram gland; second, 5 grams per kilogram gland	10 grams per kilogram gland

method. A highly potent product is obtained but the yield of hormone is somewhat less than in the standard procedure.

A summarized comparison of the standard method with the "half" method is given in table 9. Typical assay data are given in table 10.

d. Further purification of the extract

The problem of further purification of the adrenal cortical hormone has been studied with three different fractionation procedures: (1) distribution between an immiscible solvent and aqueous alkali, (2) distribution between an immiscible solvent and aqueous acid and (3) fractionation with organic solvent mixtures.

TABLE 10

Assay on whole gland extract (half method)

Extract HM-2; 1 cc. = 40 grams gland; 1 cc. = 2.0 mgm. solids.

Dog 25

WEIGHT	DAILY DOSE PER KILOGRAM			DAYS	UREA N	CLINICAL CONDITION
	Volume	Solids	Gland			
kgm.	*cc.*	*mgm.*	*grams*		*mgm. per 100 cc.*	
12.3	0.2	0.4	8	7	23–29	Normal
13.3	0.1	0.2	4	7	28–31	Normal
13.8	0.05	0.1	2	10	31–34	Normal
14.0	0.025	0.05	1	7	31–36	Normal
13.5	*0.0125*	*0.025*	*0.5*	7	31–36	Variable appetite
12.9	0.06	0.012	0.24	5	35–90	Definite insufficiency

Yield per kilogram gland = 2,000 D.U.

Contrary to earlier findings on the adrenalectomized cat, assay data on adrenalectomized dogs show clearly that the cortical hormone is rapidly destroyed by washing an ether solution of an active fraction with 0.05 N sodium hydroxide. As far as we can determine the hormone is stable toward 0.05 N hydrochloric acid under similar conditions. The cortical hormone is very rapidly destroyed by saponification with sodium ethylate at room temperature. Cortical extracts prepared with permutit fractionation give a negative biuret, ninhydrin, Hopkins-Cole, Molisch, Pauly and Liebermann-Burchard reaction. They gave a positive xanthoproteic, Millon's, alkaline copper, and alkaline phosphotungstate reaction. These positive reactions can be readily accounted for by the presence of traces of phenolic decomposition products of adrenalin. The hormone is thermolabile.

One of the most potent fractions thus far obtained was prepared by hexane fractionation of the permutit fraction. This material assayed about 50 D.U. per milligram. Typical assay data on this fraction are presented in table 11.

10. PHYSIOLOGICAL STUDIES WITH THE CORTICAL HORMONE

a. Present status of the problem of function

At the outset of this discussion the statement was made that the function of the adrenal cortex is a mystery. And so it is! It is a sad commentary upon our knowledge of the physiology of the cortex to say that, despite the fact we have a highly potent preparation of the cortical hormone which will restore to normal within 72 hours or less, comatose cats and dogs, and revive human patients in the crises of Addison's disease, we still are wondering how the hormone accomplishes the work that it does. In the case of the other endocrine organs some knowledge of the function of the gland in question was known, or strongly suspected, before a potent extract was secured. Such is not the case with the adrenal cortical hormone.

TABLE 11

Assay of hexane-soluble fraction

Extract No. 1532B: 1 cc. = 40 grams whole gland; 1 cc. = 1.5 mgm. solids.

Dog 12

WEIGHT	DAILY DOSE PER KILOGRAM			DAYS	UREA N	CLINICAL CONDITION
	Volume	Solids	Gland			
kgm.	*cc.*	*mgm.*	*grams*		*mgm. per 100 cc.*	
8.4	0.2	0.3	8	7	23–28	Normal
9.0	0.1	0.15	4	7	27–29	Normal
9.1	0.05	0.075	2	10	27–35	Normal
8.7	0.025	0.037	1	10	26–31	Normal
8.7	*0.0125*	*0.019*	*0.5*	10	29–38	Slightly less active
8.1	0.006	0.009	0.24	4	29–50	Definite insufficiency

Yield per kilogram gland = 2,000 D.U.

b. Non-effect of large doses of hormone upon the blood picture and respiratory metabolism of normal animals and man

Early in the work we were of the opinion that since we had the hormone, it should not prove very difficult to determine something about how it works. We had in mind, of course, the question of overdosage phenomena. It seemed likely that by injecting relatively huge quantities of highly potent hormone into either normal or adrenalectomized animals and following the respiratory metabolism, blood chemistry, and urine changes, that some factor or factors would alter under treatment to such a degree that a clue to function might come to light. Unfortunately the problem has not proven so simple, for the reason that overdosage phenomena are not produced by relatively enormous quantities of highly purified and concentrated cortical hormone, injected subcutaneously, intraperitoneally or intravenously into normal or adrenalectomized animals.

In collaboration with Doctors Harrop and Weinstein of the Johns Hopkins University extensive experiments were made of the blood concentration and urinary excretion of various inorganic substances and of the nitrogenous compounds, following the injection of cortical extract into normal dogs. The animal experimentation was carried on at the Johns Hopkins University by Doctors Harrop and Weinstein and the extract was supplied for the work by the Princeton Laboratory. Only a joint preliminary report of this work has so far appeared but Doctors Harrop and Weinstein have kindly permitted us to include tables 12, 13 and 14 in this paper in advance of the publication of the work *in extenso*. Responsibility for the data obtained is borne by Doctors Harrop and Weinstein while the responsibility for the potency and purity of the extracts employed is borne by the writers.

In the normal animals we have been entirely unable to detect characteristic changes in the blood constituents which we have followed. Carbon dioxide content and capacity (alkaline reserve), oxygen capacity, non-protein and urea nitrogen, creatinine, sugar, calcium, potassium, magnesium, cholesterol, lactic acid, plasma chlorides, hematocrit, and plasma proteins examined in arterial blood samples are not altered in any definite or quantitative manner. The determinations have been made at hourly intervals following injection, up to five hours, and at the end of twenty-four and forty-eight hours.

Stress should be laid, we believe, on the fact that we have used trained animals at rest; strictly in fasting conditions. There was no change in the respiratory metabolism (Oxygen consumption, or R.Q.) in a normal dog so injected within five hours or at the end of twenty-four hours. The amount of extract injected has varied from 1 to 7 cc. per kilogram of body weight. One animal received a single intravenous injection of 100 cc. of extract and showed no changes. Recently we have injected daily 15 cc. of highly potent cortical extract per kilogram of body weight into a normal cat for seven days without noticeable effect.

TABLE 12

Effect of the cortical hormone on blood constituents of normal dog

Dog 8

	NON-PROTEIN NITROGEN	CREATININE	SUGAR	PHOSPHATES AS P	CHOLESTEROL	TOTAL PROTEIN	A/G RATIO	HAEMATOCRIT CELL MASS	RED BLOOD CELLS	OXYGEN CAPACITY
	mgm. per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*grams per cent*		*parts in 10*	*mil-lions*	*volume per cent*
October 8, 1930:										
9:00 a.m.	30	1.3	100	2.7	130	6.25		4.4	5.42	18.65
1:05 p.m.	26	1.4	95	3.3	111	5.20		4.0	5.34	18.10
25 cc. intravenous injection										
2:05 p.m.	22	1.3	95	3.3	120	5.98	34/66	4.0	5.30	17.65
3:05 p.m.	26	1.3	97	3.4	111	5.36	41/59	4.1	5.33	17.82
4:05 p.m.	24	1.3	95	3.4	103	5.34		4.14	5.31	17.60
5:05 p.m.	21	1.3	90	3.9		5.51		4.0	5.34	16.90
October 9, 1930:										
9:00 a.m.	22	1.3	75	2.7	83	5.50	42/58	4.07	5.67	17.77

Some of the pertinent data are given in tabular form below. The extract injected assayed 4 to 10 dog units per cubic centimeter and represents our earlier material. At the time the experiments were performed, we had not yet devised methods for increasing the potency of the extract to the 40 to 80 D.U. level. However, considering the large dosage employed and the fact that identical material was successfully used by Rowntree in the crisis of Addison's Disease, we believe the results obtained with normal dogs to be characteristic of the adrenal cortical hormone. The data listed in tables 12 and 13 are self-explanatory. Dog 8 received 25 cc. of the extract intravenously and dog 4 received 100 cc. by vein. The results are essentially negative. The experiments were repeated on two other animals with similar findings in both cases. In all of the dog experiments the blood samples were obtained from the femoral artery or left ventricle to avoid any stasis effect.

TABLE 13

Effect of the cortical hormone on blood constituents of normal dog

Dog 4

	NON-PROTEIN NITROGEN	CREATININE	SUGAR	PHOSPHATES AS P	CHOLESTEROL	TOTAL PROTEIN	A/G RATIO	HAEMATOCRIT CELL MASS	RED BLOOD CELLS	OXYGEN CAPACITY	WEIGHT
	mgm. per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*grams per cent*		*parts in 10*	*mil-lions*	*volume per cent*	*pounds*
October 10, 1930:											
9:00 a.m.	40	1.4	85	4.1	78	4.77		4.09	5.78	17.32	
1:15 p.m.	35	1.4	90	3.4	82	4.89		3.70	5.08	16.42	
100 cc. intravenous injection											
1:40 p.m.	36	1.4	90	4.1	81	4.45		3.90	5.01		
2:30 p.m.	36	1.4	95	4.1	83	4.36		3.4	4.7	15.53	
3:30 p.m.	33	1.4	92	3.9	76	4.82		3.4	4.7	15.09	
4:40 p.m.	38	1.4	95	4.1	50	4.4	46/54	3.5	5.2	14.97	
9:15 p.m.	32	1.4	95	4.3	57	4.1		3.5	4.9	15.09	
October 11, 1930:											
9:00 a.m.	33	1.4	95	3.3	53	4.47		3.5	4.91	15.34	32¼
October 16, 1930	66	1.5	85	5.5	115	4.79		3.2		15.79	
October 22, 1930	32	1.4	80	3.6	150	5.00		3.79		17.70	32
October 28, 1930	70	1.5	76	3.2	106	4.35		4.05		16.95	29¾
November 5, 1930	26	1.5	75	3.7	150	5.52	50/50	3.34		16.55	31

Several experiments were performed upon normal human subjects. The extract was injected intravenously in 20 to 30 cc. doses and the blood samples for analysis drawn at varying periods following injection. We regard the results as comparable with those obtained with dogs. Significant changes in the various blood constituents studied were not induced.

Studies of the respiratory metabolism were also made on both dogs and human subjects along with the blood constituents. In none of the experiments were significant changes encountered.

TABLE 14

Effect of adrenal cortical hormone on blood constituents of normal human subjects

NAME OF SUBJECT, C. B.	NON-PROTEIN NITROGEN	CREATININE	SUGAR	PHOSPHATES AS P	CHOLESTEROL	TOTAL PROTEIN	A/G RATIO	HAEMATOCRIT	RED BLOOD CELLS	OXYGEN CAPACITY	WHITE BLOOD CELLS
	mgm. per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*grams per cent*				*volume per cent*	
October 13, 1930:											
2:40 p.m.	30	1.3	85	2.6	244	5.36	68/32	32.3		16.80	
20 cc. intravenous injection											
3:20 p.m.	28	1.4	85	2.6	230	5.28		31.0		16.26	
4:30 p.m.	28	1.5	88	2.9	230	5.82	61/39	33.1		17.72	
5:00 p.m.	Supper										
6:55 p.m.	28	1.4	140	2.8	200	5.36		31.8		16.42	
SUBJECT, J. O.											
October 15, 1930:											
1:10 p.m.	31	1.5	100	3.7	150	6.14		39.2	4.54	20.15	7,400
20 cc. intravenous injection											
1:50 p.m.	35	1.5	100		166	6.43		39.0	4.36	19.52	9,080
4:00 p.m.	26	1.4	102	3.7	176	6.66		39.0	4.72	19.45	9,120
9:20 p.m.	34	1.5	95	3.4	166	5.76		38.5	4.86	19.39	
October 16, 1930:											
9:00 a.m.	30	1.5	102		187	6.28		39.0	4.91	19.45	
October 17, 1930:											
1:30 p.m.	23	1.3	102	3.6	176	6.38		37.5	4.93	18.87	6,080
30 cc. intravenous injection											
3:15 p.m.	36	1.3	102	3.6	176	5.97		37.8	4.78	18.95	9,380
5:00 p.m.	Supper										
8:15 p.m.	34	1.3	90	3.6	166	6.05		39.0	5.02	18.49	7,400

Our studies on normal dogs treated with relatively huge quantities of the cortical extract, convince us that there is no such thing as overdosage with this hormone—at any rate in so far as respiratory metabolism and the usual blood and urinary constituents are concerned. This fact has been a great disappointment to us, since the work on separation of the hormone has not as yet greatly aided in picking up a clue as to function. With respect to overdosage, the cortical hormone differs from adrenalin, insulin, parathyroid and thyroxin, but falls into the same class as the anterior pituitary and gonad hormones, which also do not have toxic effects in normal animals.

c. Studies on respiratory metabolism

1. Adrenalectomized and thyroidectomized cats and adrenalectomized dogs. In our early work, we were much impressed with the fall in body temperature of adrenalectomized cats following bilateral adrenal removal when the animals are showing marked symptoms. It is the usual thing for the rectal temperature of adrenalectomized cats to fall steadily from a normal temperature of 101.5 to 102°, to 93° or lower when the animal is verging on prostration. Following administration of adequate dosage of cortical hormone, the rectal temperature steadily rises to normal over a period of 24 to 36 hours, when all symptoms disappear.

In collaboration with Dr. Bruce Webster of the Department of Medicine, College of Physicians and Surgeons, Columbia University, we attempted to determine the effect of bilateral adrenalectomy on the respiratory exchange of cats, together with the changes in metabolism produced by the administration of the cortical hormone to these adrenalectomized animals.

The animals were operated in 2 stages in our experiments. Healthy adult animals were used. They were maintained on a constant diet and were fed approximately 20 hours before the beginning of the metabolism determination. The respiratory exchange was measured by Marine's modification of Haldane's open circuit apparatus for two-hour periods. Injections of the cortical hormone were made at least 15 hours prior to the beginning of the determination of metabolism. Dissected cortex extract was used. The adrenalin content of this extract was between one part in 1,500,000 and one part in 2,000,000. The injections were made subcutaneously.

Previous observers have shown that the removal of one adrenal does not alter the level of metabolism. Following the removal of the second adrenal gland, there was no appreciable change in metabolism for from 4 to 6 days in our series of animals. From the sixth to the tenth day there was a progressive fall until a level approximately 50 per cent below normal was reached when the animal was in a prostrate condition. Following the administration of cortical hormone to these prostrate animals, an increase in metabolism occurred within 24 to 48 hours. In from 48 to 72 hours after the beginning of the administration of the hormone, the metabolism had risen, in each case, to a level varying between 10 and 18 per cent above the normal previously established for that individual animal. At this point the administration of hormone was stopped and there was a fall within 24 to 36 hours to the normal level. This was maintained for a varying time period (several days), during which the animal appeared normal. At the end of that time the metabolism began gradually to decrease, signs of adrenal insufficiency developed, and the cycle was repeated.

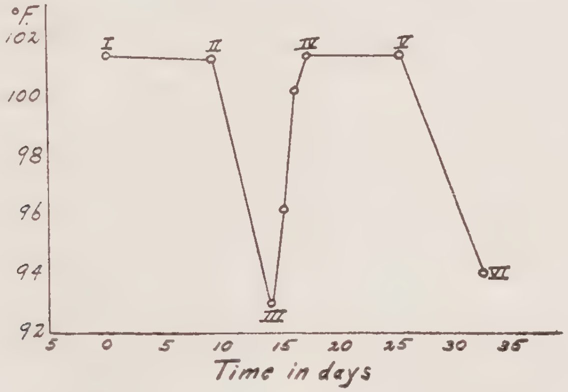

Fig. 9. Rectal Temperature Chart of a Bilaterally Adrenalectomized Cat Treated Intraperitoneally With Cortical Hormone

I, second gland removed; II, no symptoms; III, prostrate; treatment begun; IV, no symptoms; V, treatment discontinued; VI, prostrate (died of adrenal insufficiency).

The changes have been studied in a series of 6 cats. One was observed through 3 cycles, one through 2 and the remainder through one. The metabolic changes were essentially the same in each case.

Our next step was to test the effect of administration of the cortical hormone to normal animals. Cortical hormone was injected subcutaneously in quantities of from 6 to 15 cc. daily into normal cats for periods varying from 2 to 10 days. Measurements of the respiratory exchange were made 18 hours after the injections. In a series of 12 such experiments, no appreciable constant variation from normal occurred. Further, the general health of the animals did not appear to be affected. Similar experiments were carried out on three normal rabbits with essentially the same result.

The normal level of respiratory metabolism was determined in a series of five cats. Total thyroidectomies were then performed. The metabolism gradually fell to a level varying between 20 and 25 per cent below normal. The animals gained in weight and showed general symptoms of thyroid insufficiency. At this point the cortical hormone was injected subcutaneously in quantities varying from 10 to 50 cc. over a time period of from 1 to 3 days. Within 24 hours after the injection there was an increase in respiratory metabolism of from 15 to 30 per cent. This change occurred in 80 per cent of the experiments and continued for from 24 to 48 hours after the administration of hormone was discontinued.

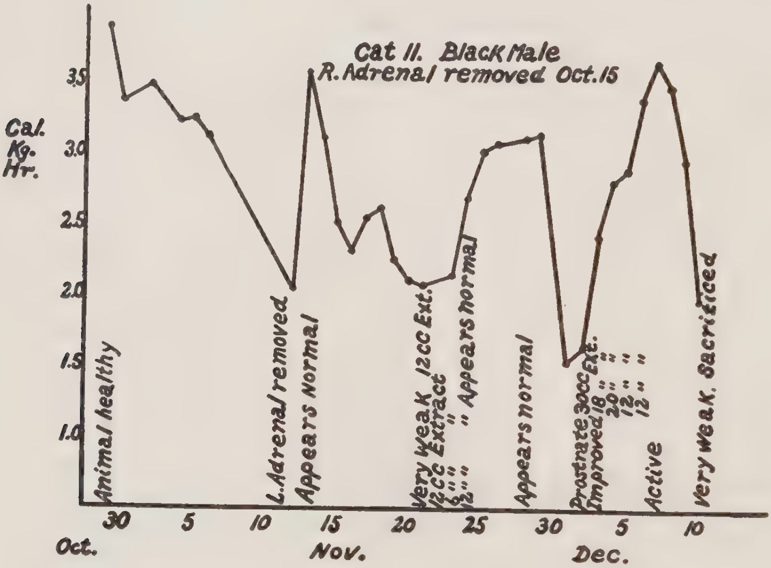

FIG. 10. EFFECT OF THE CORTICAL HORMONE ON THE HEAT PRODUCTION OF A BILATERALLY ADRENALECTOMIZED CAT

In 20 per cent of the experiments, no appreciable changes in metabolism occurred after injection of the cortical hormone. Further, once an elevation of metabolism had been obtained and the animal allowed to return to the myxedematous level, a second rise could not be obtained until a period of 10 to 14 days had elapsed. At present we are unable adequately to account for the variability of this reaction. Figure 11 shows a typical case where elevation of metabolism of a myxedematous cat followed injection of the cortical hormone.

It was considered advisable to determine whether or not the thyroid gland was a factor in bringing about the elevation of metabolism which occurred when the adrenal cortical hormone was injected into bilaterally adrenalectomized cats. Accordingly, the previously thyroidectomized cats used in the above experiments were bilaterally adrenalectomized in 2 stages. The respiratory exchange was measured daily. As was the case in the animals in which the thyroid was intact, the metabolism fell with the development of symptoms of adrenal insufficiency. When these symptoms became severe, injections of cortical hormone were begun. The metabolism rose promptly to a level slightly above the normal and remained there as long as the cortical hormone was administered. In other words, the changes in the

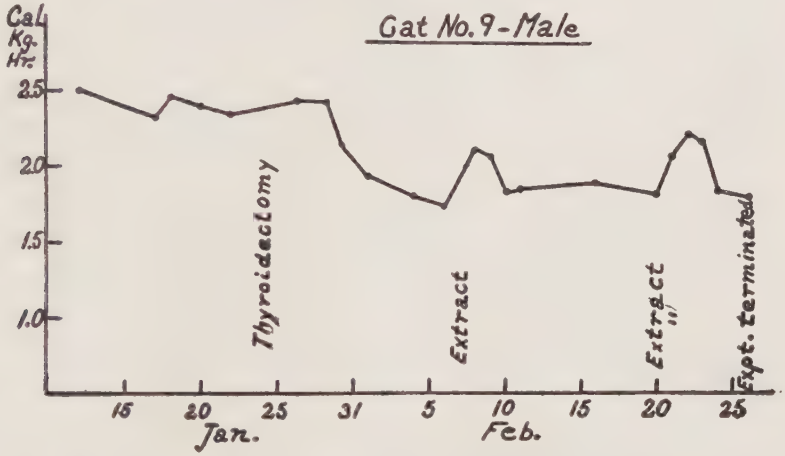

FIG. 11. EFFECT OF THE CORTICAL HORMONE ON THE HEAT PRODUCTION OF A THYROIDECTOMIZED CAT AT THE MYXEDEMATOUS LEVEL

respiratory exchange following bilateral adrenalectomy in cats are essentially the same whether the thyroid is intact or totally removed and the results indicate that the adrenal cortical hormone has a direct action upon respiratory metabolism. Figure 12 shows the effect upon metabolism of administering the cortical hormone to a thyroidectomized-adrenalectomized cat. The quantity of extract given daily varied from 15 to 21 cc. per day.

In collaboration with Doctors George Harrop, and A. Weinstein of the Department of Medicine, Johns Hopkins University, we undertook studies of the respiratory metabolism of adrenalectomized dogs. The conception and execution of this work and the data obtained are the sole responsibility of Drs. Harrop and Weinstein. A drop in respiratory metabolism was a constant observation following onset of adrenal insufficiency in these animals. In animals which subsequently recover following injection of adequate amounts of cortical hormone, the oxygen consumption may drop at the time of maximum insufficiency to 20–25 per cent below the normal value, at which time the R.Q. also has fallen to 0.72 to 0.71. Following administration of

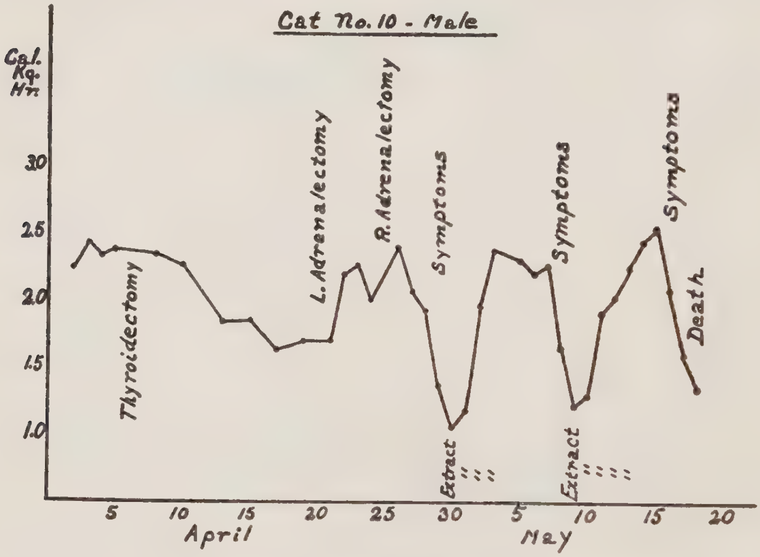

FIG. 12. EFFECT OF THE CORTICAL HORMONE ON THE HEAT PRODUCTION OF A THYROIDECTOMIZED ADRENALECTOMIZED CAT

extract and the return of appetite the respiratory metabolism usually returns slowly and the R.Q. more rapidly to their original normal levels.

The respiratory metabolism of the adrenalectomized dog apparently does not sink to as low a level as occurs in the adrenalectomized cat. This is probably a species difference in the reaction between the two forms to acute cortical insufficiency. Rarely does one observe rectal temperatures below 97° in dogs dying from adrenal insufficiency, whereas in cats presenting equally severe symptoms, the rectal temperature frequently sinks below 90°, and is invariably far lower than that found in the dog. The condition observed in adrenalectomized rats is similar to that found in cats.

2. Addison's disease patients. It is of interest in this connection to quote from a recent paper by Greene, Rowntree, Swingle, and Pfiffner on the effect of treatment with the cortical hormone on the metabolism of patients with Addison's disease.

> The effect of treatment with the cortical hormone on the metabolism of patients with Addison's disease is variable. There is very little change in patients whose metabolic rate is normal. When the metabolic rate of the patient is reduced, treatment causes elevation toward, although not always to within normal limits. Thus, in case 12 the change was from -31 to -13 per cent and in case 16, from -22 to -4 per cent. The maximal increase was from -18 to $+12$ per cent, and in no case did the basal metabolic rate after treatment rise above $+14$ per cent. The increase in the basal metabolic rates of these patients may in part be associated with the increased strength and vitality of the patient, but this is not ncessarily the case, for we have observed marked clinical improvement associated with considerable gain in weight without any corresponding change in the metabolic rate.

Figure 13 shows the effect of the cortical hormone upon the basal metabolic rate of Addison's disease patients.

> That the change in the respiratory exchange is not an accurate guide to the clinical condition is also indicated by three cases in which there were considerable increases in metabolic rate, with only a questionable degree of clinical improvement.
>
> The study of the respiratory exchange of this group of patients shows that the clinical features are in harmony with the basal metabolic rate in

the presence of suprarenal insufficiency, and in demonstrating improvement following treatment with cortical extract. The results with patients, however, are much less marked than are those with animals, and do not serve to determine whether these changes are direct results of the suprarenal insufficiency or whether they are secondary to the associated nutritional disturbance.

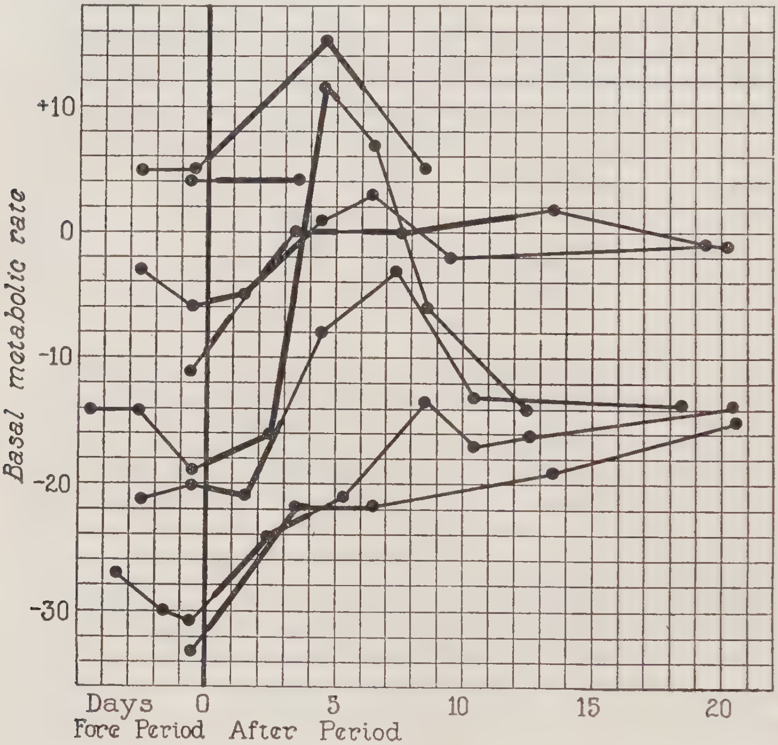

Fig. 13. Changes in the Basal Metabolic Rate of Patients with Addison's Disease Treated with the Suprarenal Cortical Hormone

The days on which treatment was given are marked by the heavy line

However, it must be remembered that the picture in Addison's disease is complicated by other factors which are lacking in the experimental animals, and which render the results obtained by metabolic studies on human patients difficult to interpret. Our studies upon adrenalectomized cats and dogs, which were at all times carefully controlled, lead us to believe that the changes in respiratory metabolism following adrenal extirpation and following replacement therapy with the cortical hormone, are direct results of the suprarenal insufficiency, and of the activity of the hormone. In other words, the adrenal cortical hormone appears to have a direct effect upon the respiratory metabolism of mammals.

d. The renal failure of adrenal insufficiency

Probably the most interesting result of our studies of adrenal insufficiency, is the evidence pointing to renal failure, in animals deprived of their adrenals, and the prompt restoration of kidney function to normal following administration of the cortical hormone. The discussion which follows is based upon work by Doctors Harrop and Weinstein of the Johns Hopkins Hospital and the writers. The results have already appeared in preliminary form. The experimental data were obtained by Doctors Harrop and Weinstein and the extract used and some of the adrenalectomized dogs were supplied by the writers.

It is a constant finding in our adrenalectomized dogs, that when the bilaterally operated animal, in a good state of nutrition and with well healed wounds and without infection, is deprived of adequate injections of cortical hormone, either abruptly or by gradual reduction of dosage to less than $\frac{1}{6}$ or $\frac{1}{8}$ of a cubic centimeter per kilogram of body weight daily, the first significant change we have observed is a rise in blood non-protein nitrogen and urea. (The quantities of extract refer to the original cortical extract prepared from dissected cortex which contained only 4 to 10 dog units per cubic centimeter.) This is coincident with, or may precede by a few hours, the refusal of food, and definitely precedes the drop in respiratory metabolism. Changes in blood creatinine do not occur until the animal is very ill. The serum potassium concentration rises steadily during the period of insufficiency. The secretion of urine diminishes markedly and the animal may become completely anuric. There is suppression of urinary nitrogen and urea. The chloride and inorganic phosphate excretion is suppressed when the animal begins to refuse food, and if the insufficiency is allowed to go to the point where urinary secretion is very low or almost suppressed, there is a diminution of creatin and creatinine excretion as well as that of injected phenolsulphonephthalein. In the earlier stages of insufficiency, however, suppression of total nitrogen and urea nitrogen occur before the excretion of creatinine and creatin lessens. No microscopic changes are found in the urine, but small amounts of albumin are quite regularly present during insufficiency. Lowering of systolic blood pressure does not usually occur until after the nitrogen retention has become well established.

Injection of the cortical hormone at the height of the insufficiency is then followed by a diuresis which may last for 48 to 96 hours and is accompanied by increased excretion of urinary nitrogen and urea, and of chlorides. At the same time all symptoms of insufficiency disappear. The appetite returns and the animal gains rapidly in weight.

These changes precede or approximately parallel the return of the blood non-protein nitrogen and urine excretion nitrogen to their previous values. The fall in serum potassium concentration parallels that of the non-protein nitrogen. Studies of the urea clearance of adrenalectomized dogs have not been of value in our hands, but Dr. Harrop reports that the urea clearance of some patients with Addison's disease is low. He feels that this may be a circulatory effect, however, due to a lessened blood supply to the renal parenchyma.

The idea that a functional relationship may exist between the adrenals and the kidney of mammals, is not new. Marshall and Davis in 1916 advanced the theory for the first time and based their view upon the observation that the blood urea of adrenalectomized cats steadily rises throughout the period of insufficiency. They were able to demonstrate to their own satisfaction, that a fall in blood pressure did not occur until after the nitrogen retention had been well established.

Our more recent work on dogs demonstrates that renal insufficiency is a constant finding after adrenal ablation, and that complete restoration of kidney function follows administration of the cortical hormone, with outpouring of urinary nitrogen, chlorides, phosphates and other waste metabolites. Significant anatomical lesions have not been demonstrated in the kidneys of animals dying from adrenal insufficiency so it is evident that the renal lesion, if such is present, is a functional one and occurs in the absence of demonstrable anatomical injury.

e. Heat, pregnancy and lactation in adrenalectomized dogs

A considerable literature has accumulated, especially on the clinical side, pointing to the possibility of an interrelation between the hormone of the adrenal cortex and the sex glands. The experimental data on this subject are extremely meager, in fact practically non-existent. However the facts may eventually turn out to be, our own evidence indicates clearly that if the cortical hormone does have any relation to sex, pregnancy or lactation the active substance is contained in our extracts. The following experiment illustrates the point:

An adrenalectomized dog on minimum dosage of extract, and so maintained for a period of four months, was bred to a female which had also been adrenalectomized for four months and kept normal by daily injections of small doses of hormone. The bitch became pregnant and gave birth to a litter of six healthy pups. The pups were suckled and weaned in normal fashion. After weaning the litter, extract was withheld from both parents and they promptly developed severe symptoms of adrenal insufficiency. The experiment demonstrates that the extract contains everything necessary for maintaining all physiological functions of the organism, including sex urge, pregnancy and lactation. Figure 8 is a picture of the male and female dog used in the breeding experiment. Figure 14 shows the mother nursing her young. At the present writing we have several dogs, both male and female, in the laboratory, bilaterally adrenalectomized and main-

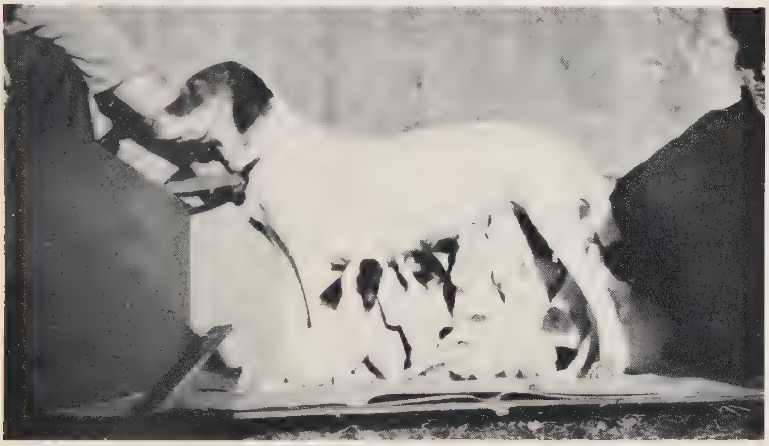

Fig. 14. Bilaterally Adrenalectomized Dog Maintained on Extract for Several Months before Passing through Normal Periods of Heat, Pregnancy and Lactation

tained in normal condition for a year. We employ the males for breeding purposes regularly.

f. Conclusion

In the present state of our knowledge of the adrenal cortex, it is impossible to draw any definite conclusions regarding function. We have pointed out several times in this paper that the real functional significance of the adrenal cortical hormone is unknown. All of the changes reported as occurring in the organism following bilateral adrenalectomy, we regard as secondary to some at present unknown, underlying derangement of the animal. To our knowledge no one has yet succeeded in presenting definite, clear-cut unequivocal evidence of cortical function. The literature is filled with theories and hypotheses of adrenal function—on the relation of the cortex to lipid, carbohydrate, tissue metabolism and mineral metabolism. However, it is our candid opinion that none of these theories or hypotheses withstand careful scrutiny and experimental test, nor do they throw any new light upon the mystery of cortical function. The function of the adrenal cortex is a subtle and intriguing one, but the solution is not yet at hand.

11. USE OF THE CORTICAL HORMONE IN TREATMENT OF ADDISON'S DISEASE

Since neither of the writers are physicians, the work of clinical testing of our adrenal cortical preparations was placed in the competent hands of Drs. Leonard Rowntree and Carl Greene of the Mayo Clinic. We wish to express our gratitude to these investigators for their painstaking efforts, kindly coöperation, and keen interest in the work. The extract used by these workers up until June, 1931 was supplied by the Princeton Laboratory. At that time we considered the preliminary clinical tests in Addison's disease as satisfactorily completed and discontinued all further work along this line. The following discussion of the clinical data is a resumé prepared from the published reports of Doctors Rowntree, Greene, Swingle, and Pfiffner.

a. Résumé of the clinical findings on Addison's disease patients treated at the Mayo Clinic with cortical hormone

When both suprarenal glands are removed from an animal, whether by means of an operation in one stage or in two stages, suprarenal insufficiency becomes complete with the stroke of the surgeon's knife. The subsequent clinical course is rapidly downward to a fatal termination. The course of the patient with Addison's disease shows many points of similarity, yet the differences are characteristic and fundamental. For the purpose of this consideration the course may arbitrarily be divided into three periods. The distinction between these periods is not always sharp, but they usually are recognizable.

The three periods in the course of Addison's disease are:

The period of initial destruction of the suprarenal glands. Approximately two-thirds of all cases of Addison's disease are due to tuberculosis of the suprarenal glands. One-fifth of the total number are due to atrophy, particularly of the cortex. Experimental studies have disclosed that removal of one suprarenal gland may be compatible with life of the animal and that bilateral suprarenalectomy is not necessarily fatal if accessory glandular tissue is present. The suprarenal glands in many cases of Addison's disease in which there is a history indicating duration limited to a few weeks, are represented by large caseous or calcareous masses suggestive of a chronic, slowly growing lesion. These observations all suggest that the suprarenal glands possess considerable functional reserve and that clinically recognizable signs of Addison's disease do not appear until this reserve is seriously impaired or destroyed. When the functional activity of the glands becomes insufficient for the daily physiologic requirements of the body, the second stage is initiated.

The period of the recognizable clinical syndrome. The characteristic clinical symptoms of Addison's disease, namely, asthenia, pigmentation, loss of weight and strength and arterial hypotension, are well known. Once they appear the diagnosis is established. Although the efficiency and activity of the patient may be greatly reduced, disability is not necessarily complete. The average length of life following the appearance of the clinical symptoms of Addison's disease is 16.5 months, according to Rowntree, and 13.3 months according to Guttman, although Guttman reported 11 cases in which the duration was more than 5 years. A greater or lesser degree of suprarenal insufficiency must be present during this period, yet the patient is in a fairly satisfactory clinical condition. When we mention the chemical and metabolic changes in Addison's disease, it is to this period that we refer.

The terminal period or crisis. Patients with Addison's disease are exceedingly susceptible to intercurrent infection, and usually are sensitive to fatigue and minor traumas, such as dental extraction. Such predisposing factors frequently initiate an acute, shock-like condition, or a period of crisis, although the latter may also develop spontaneously. This condition is characterized by a severe gastrointestinal upset, nausea and vomiting, pain, circulatory collapse, low blood pressure, cold extremities, and lowered bodily temperature. The urinary output is often reduced and the respiration may have the characteristic irregularity described by Rowntree.

The outcome of such a crisis varies with the severity of the condition. In some cases several such upsets have occurred from which the patients have recovered spontaneously. In other cases, treatment with adrenal cortical hormone is strikingly beneficial. Still other patients are refractory to treatment, and the crisis is terminal.

The chemical and metabolic determinations in Addison's disease will be presented on the basis of the last two periods. The change from the one to the other may be insidious, and there is room for difference of opinion so far as the precise severity of symptoms necessary for the establishment of a crisis is concerned, but in general the distinction between the two groups is permissible.

The results of administering cortical extract to patients in the crisis of Addison's disease is very striking and such patients generally are quickly restored to apparently normal health and activity; the restoration in some cases being quite dramatic. This, of course, is what one would expect from the results upon revival of comatose adrenalectomized cats and dogs. The Addison's disease patient in collapse and crisis is the human counter-part of the dog or cat in the crisis of acute adrenal insufficiency following gland extirpation. There are differences between the two conditions to be sure, such as the bronzing of the skin in Addison's disease, which is lacking in the acute suprarenal insufficiency of animals, and consistently low blood pressure, which may be a terminal phenomenon in animals. Other symptoms are quite similar, e.g. weakness, muscular asthenia, weight loss, anorexia, gastrointestinal disturbances, and dehydration.

The clinical changes observed in patients responding satisfactorily to treatment with the cortical hormone are:

1. The disappearance of anorexia, nausea and vomiting, and the reappearance of appetite and the development of the sense of hunger.
2. Relief from fatigue and an increase in strength and endurance.
3. Disappearance of pains and an improvement in sleep.
4. Decrease in pigmentation.
5. A gain in weight.
6. Return of normal gastrointestinal activity.
7. A change in mental attitude characterized by hope and euphoria.
8. A moderate increase in blood pressure when the patient was in crisis or shock, but very little change when the patient was in good condition.
9. An increased resistance to infection, or to the effects of surgical procedure.
10. Partial or complete rehabilitation of the patient with a return to work.

The most constant and striking effect is the disappearance of anorexia, nausea and vomiting and also, peculiarly enough, the distressing pain in the loins that is present in so many of these patients. The sensation of hunger returns and the patient begins to take food. Normal intestinal activity is reestablished. The patients lose their sense of fatigue and often from the beginning they enjoy profound and prolonged sleep, awaking with a refreshed sense and the inquiry as to whether they have been given anything in the way of a hypnotic. Increase in strength and endurance appears early and is marked, and out of all proportion to the objective findings. With the taking of food, the gain in weight is remarkable (as much as 9½ pounds in 8 days). This is due mostly to the taking of food but may in part result from an increase in the store of water in the body.

There is a decided decrease in pigmentation which becomes apparent within three or four days.

One of the patients on admission was looked upon as a negro by his roommate in the hospital who objected to being in the same room with him, but after reassurance stayed and witnessed the transformation of a "negro" to a white man. The pigmentation decreases most markedly in the central portion of the face.

With the physical betterment there is a marked improvement in mental attitude and outlook. Hope returns with the falling away of depression, apprehension and fatigue. Euphoria develops in many instances.

The effect on blood pressure is slight in comparison with the other changes noted and is, we believe, rather secondary in nature. It is astounding how well some of these patients appear to be during convalescence even though there is slight or no increase in blood pressure. The increase is gradual, so that in most of our cases, both the diastolic and systolic pressures are within the lower limits of normal within one or two weeks.

An entirely new problem is coming in the offing. With the improvement in the clinical condition there is a return of normal interest and a desire to return to occupations. With other methods of treatment the patients have been content to remain invalids, but the physical and mental improvement following the administration of the cortical hormone has led in many instances to the request to return to work. Because of the inadequate supply of the hormone, and also of our lack of knowledge as to the permanency of the effect, these requests have been denied to date.

Among the most striking phenomena observed have been the increase in resistance to infection, the ability to withstand surgery and the disappearance of idiosyncrasies to the actions of drugs, so commonly encountered in patients with Addison's disease. The majority of patients with Addison's disease succumb rapidly and without resistance to intercurrent infections. In one instance we have observed a patient under treatment with the cortical hormone pass through an infection which we have every reason to believe would have been immediately fatal in the absence of treatment. Recently similar results were obtained with another patient who withstood in excellent fashion a major surgical operation.

b. Nitrogen metabolism

We have studied the partition of urinary nitrogen and the approximate nitrogen balance in 9 cases of Addison's disease, before, during, and after treatment with the suprarenal cortical hormone. Because of the anorexia and gastrointestinal distress that accompanies many cases of untreated Addison's disease, it has not been possible to put these patients on a constant, rigid diet. It has been necessary, instead, to allow the patient to eat as desired, but all food served and any uneaten portion was weighed. In this way a record was kept of all food actually consumed. The content of nitrogen and calories was then calculated from the Atwater-Bryant tables. The stools were not saved, but in calculating the nitrogen balance, the fecal nitrogen was assumed to be one-tenth of the intake. Care was taken in the collection of urine, and the periods over which the daily specimens of urine were collected were, for the most part, accurate to within 15 minutes. The analytic methods of Folin were used throughout in urinalysis.

The analytic results in illustrative cases are given in tables 15, 16 and 17. For the most part, the composition of the urine corresponded to the lower level of the usually accepted normal standards. The total nitrogen varied between 5.30 and 11.77 grams for 24 hours. Urea was the major constituent. The values for the creatinin nitrogen, uric acid nitrogen, aminoacid nitrogen and rest nitrogen all fell within the limits of normal, and to this extent served to exclude disturbances in endogenous metabolism. There was no change in these values with treatment. The acid-base balance, as indicated by the hydrogen-ion concentration, the titratable acidity, the ammonia nitrogen or the excretion of phosphate was likewise normal, and was not significantly changed during treatment.

We have found a considerable degree of creatinuria (more than 30 mgm. of creatin nitrogen daily, or more than 10 per cent of the preformed creatinin) in 7 of 13 cases studied. When creatin has been present it has been reduced in amount or has disappeared completely as a result of treatment with the cortical hormone. Especial mention should be made of the excretion of urea in the ninth case in our experience. This patient was weak, but was in a satisfactory clinical condition on the first day of the metabolic study. The second day he had no appetite, ate very little, vomited once, and took only a small amount of fluid; the amount of urine was greatly reduced. Treatment with cortical hormone was started, the intake of fluid was pushed during the next two days, and the patient made a prompt and spectacular clinical recovery. The elimination of practically all urinary constituents was reduced the day the urinary output was diminished. That this was due to retention is shown by the increased excretion the 2 following

TABLE 15

The effect on metabolism of treatment with cortical hormone in the seventh case of our experience

	JANUARY												
	3	4	5	6	7	8	9	10	11	12	13	14	15
Weight, kgm.	52.4	52.4	52.8	52.4	52.4	52.6	52.6	52.8	53.8	53.4	54.0	53.8	53.8
Cortical hormone, cc.					14	21	21	20	20	10			
Intake of food:													
Total calories	1,120	1,539	1,809	1,936	1,664	1,691	1,862	2,032	2,000	2,000	2,000	2,000	2,000
Protein expressed as nitrogen, grams	6.88	6.88	6.08	8.32	6.56	6.56	8.64	10.10	9.60	9.60	9.60	9.60	9.60
Basal metabolic rate, per cent		+5							+15				+4
Urine for 24 hours:													
Volume, cc.	750	1,350	850	1,550	650	750	1,350	1,150	950	1,350	1,970	1,000	1,300
Total nitrogen, grams	6.35	7.99	5.42	7.40	3.98	6.65	5.70	4.78	5.26	5.78	5.41	3.85	5.55
Urea nitrogen, grams	4.65	5.57	3.99	5.34	2.55	4.85	4.77	3.78	4.20	4.77	4.13	2.92	4.16
Ammonia nitrogen, grams	0.25	0.46	0.19	0.27		0.28	0.21	0.18	0.23	0.19	0.25	0.12	0.24
Total creatinin nitrogen, grams	0.37	0.66	0.31	0.46	0.25	0.47	0.40	0.31	0.30	0.35	0.32	0.26	0.35
Preformed creatinin nitrogen, grams	0.27	0.48	0.24	0.37	0.22	0.39	0.36	0.27	0.28	0.34	0.31	0.25	0.34
Creatin nitrogen, grams	0.10	0.18	0.07	0.09	0.03	0.08	0.04	0.04	0.02	0.01	0.01	0.01	0.01
Amino nitrogen, grams	0.16	0.29	0.17	0.26	0.19	0.23	0.06	0.05	0.08	0.08	0.06	0.07	0.10
Uric acid nitrogen, grams	0.05	0.04	0.04	0.07	0.03	0.04		0.06	0.07	0.07		0.07	0.10
Rest nitrogen, grams	0.87	0.97	0.71	0.99	0.45	0.78	0.25	0.40	0.38	0.32	0.47	0.41	0.60
Nitrogen balance, grams	−0.16	−1.80	+0.05	+0.09	+1.92	−0.75	+2.08	+4.31	+3.38	+2.86	+3.23	+4.79	+3.09
Hydrogen-ion concentration	5.20	5.00	5.20	4.80	6.90	5.90	5.50	5.90	5.50	5.70	6.30	6.20	5.90
Total acids, cc. N/10	212	241	162	208	50	214	168	166	157	198	145	115	170
Phosphate, grams	0.40	0.51	0.34	0.39	0.25	0.44	0.34	0.31	0.37	0.36	0.30	0.28	0.34
Sodium chlorid, grams	2.73	4.12	2.72	5.22	2.81	3.71	3.72	3.33	3.99	4.19	6.60	4.52	5.89
Blood:													
Urea, mgm. in each 100 cc.	40			22									32
Sulphates, mgm. in each 100 cc.	6.7			5.1									4.9

TABLE 16

The effect on metabolism of treatment with cortical hormone in the ninth case of our experience

	JANUARY												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Weight, kgm.		54.00			54.40		54.70	55.10			56.00	55.50	55.50
Cortical hormone, cc.			15	15	15	15							
Intake of food:													
Total calories	2,500	900	700	1,500	1,250	1,950	2,650	2,400	2,650	2,850	2,450	2,600	2,700
Protein expressed as nitrogen, grams	10.40	1.60	5.60	9.60	7.20	10.40	11.20	11.20	11.20	11.20	10.40	12.00	9.60
Basal metabolic rate, per cent		+1			−7		+1		−5			−9	
Urine for 24 hours:													
Volume, cc.	1,440	780	1,500	1,520	1,050	950	1,250	1,100	1,440	1,450	1,300	1,410	1,230
Total nitrogen, grams	10.56	4.73	12.24	13.52	10.25	9.65	9.98	8.44	9.51	9.16	7.73	7.54	7.65
Urea nitrogen, grams	8.85	3.67	10.36	11.53	8.63	7.65	8.25	7.41	8.43	7.79	6.65	6.33	6.69
Ammonia nitrogen, grams	0.21	0.19	0.34	0.39	0.31	0.47	0.33	0.23	0.17	0.17	0.20	0.13	0.14
Total creatinin nitrogen, grams	0.59	0.41	0.65	0.56	0.39	0.57	0.47	0.36	0.44	0.45	0.38	0.44	0.41
Preformed creatinin nitrogen, grams	0.57	0.39	0.63	0.52	0.37	0.54	0.44	0.35	0.44	0.43	0.38	0.42	0.39
Creatin nitrogen, grams	0.02	0.02	0.02	0.04	0.02	0.03	0.03	0.01		0.02		0.02	0.02
Amino nitrogen, grams	0.05	0.04	0.07	0.05	0.04	0.06	0.05	0.05	0.05	0.05	0.04	0.05	0.05
Uric acid nitrogen, grams	0.11	0.06	0.19	0.21	0.16		0.13	0.12	0.16	0.15	0.13	0.11	0.11
Rest nitrogen, grams	0.75	0.36	0.63	0.78	0.72	0.83	0.75	0.27	0.26	0.55	0.33	0.48	0.26
Nitrogen balance, grams	−1.20	−3.29	−7.20	−5.88	−3.75	−0.29	+0.10	+1.64	+0.57	+0.92	+1.63	+3.26	+0.99
Total acids, cc. N/10	217	83	361	374	230	294	227	178	157	240	236	324	270
Phosphate, grams	0.58	0.29	0.74	0.86	0.49	0.54	0.50	0.39	0.50	0.61	0.51	0.50	0.53
Sodium chlorid, grams	6.66	2.15	7.81	9.48	6.30	4.95	7.73	9.11	12.73	12.21	10.62	9.72	9.70
Blood:													
Urea, mgm. in each 100 cc.	66		56		46					28			
Sulphates, mgm. in each 100 cc.	7.73				7.95					4.15			

TABLE 17

The effect on metabolism of treatment with cortical hormone in the fourteenth case of our experience

	FEBRUARY												MARCH					
	17	18	19	20	21	22	23	24	25	26	27	28	1	2	3	4	5	6
Weight, kgm.	57.00	57.30	57.50	57.50	57.50	57.40	57.40	57.00	57.00	56.30	56.70	57.20	57.70	58.20	58.80	59.90	60.00	60.00
Cortical extract, cc.						10	12			15	15	8	10					
Intake of food:																		
Total calories	2,800	2,800	2,800	2,700	2,700	2,800	2,800	2,800	2,300	2,800	2,750	2,800	3,200	3,000	3,150	3,650	3,650	4,400
Protein expressed as nitrogen, grams	11.20	10.40	11.20	11.20	11.20	11.20	11.20	11.20	8.80	11.20	11.20	11.20	12.80	12.80	16.00	19.40	19.40	20.00
Basal metabolic rate, per cent	−4	−7	−13	−7	−13		−9		−13	−13		−3		−3		−9		−7
Urine for 24 hours:																		
Volume, cc.	760	760	1,000	1,050	1,490	1,240	1,200	1,150	700	860	850	1,070	1,510	1,500	1,610	1,690	1,900	2,500
Total nitrogen, grams	9.25	9.25	6.42	6.30	6.00	6.03	7.36	6.98	6.14	8.80	7.80	7.74	7.77	6.66	8.29	8.44	9.11	7.91
Urea nitrogen, grams	7.68	7.68	5.12	5.05	4.46	4.76	6.17	5.80	5.19	7.66	6.74	6.33	6.29	5.15	6.69	6.73	7.30	6.53
Ammonia nitrogen, grams	0.51	0.51	0.32	0.31	0.51	0.22	0.22	0.21	0.15	0.23	0.21	0.40	0.42	0.38	0.44	0.34	0.42	0.34
Total creatinin nitrogen, grams	0.49	0.49	0.47	0.48	0.49	0.48	0.52	0.52	0.43	0.49	0.49	0.46	0.51	0.49	0.56	0.53	0.53	0.49
Preformed creatinin nitrogen, grams	0.49	0.49	0.45	0.48	0.48	0.46	0.50	0.50	0.42	0.47	0.47	0.45	0.50	0.48	0.55	0.52	0.51	0.45
Creatin nitrogen, grams			0.02		0.01	0.02	0.02	0.02	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.04

Uric acid nitrogen, grams....	0.17	0.17	0.18	0.18	0.12	0.17	0.16	0.15	0.12	0.17	0.17	0.16	0.17	0.19	0.22	0.24	0.23	0.18
Rest nitrogen, grams........	0.40	0.40	0.33	0.27	0.42	0.40	0.29	0.30	0.25	0.25	0.19	0.39	0.38	0.47	0.38	0.60	0.63	0.37
Nitrogen balance, grams...	+0.83	+0.03	+3.66	+3.78	+4.08	+4.05	+2.72	+3.10	+1.78	+1.28	+2.28	+2.34	+3.75	+4.86	+6.11	+9.02	+8.35	+10.09
Total acids, cc. N/10.........	384	384	154	201			202	203	256	378	325	472	249	240	243	199	269	323
Phosphate, grams........	0.70	0.70	0.38	0.48		0.47	0.58	0.52	0.49	0.77	0.66	0.69	0.53	0.51	0.64	0.63	0.72	0.74
Sodium chlorid, grams........	8.64	8.64	11.82	10.78		10.20	11.96	11.48	7.48	9.50	5.15	5.69	7.79	6.72	10.37	12.50	18.92	11.15
Blood:																		
Urea, mgm. in each 100 cc....	32													22				
Sulphates, mgm. in each 100 cc.	3.20																	

days. This is well shown by the figures for urea, creatinin and titratable acidity. The output of total nitrogen the second day of the experiment was 5.83 grams less than on the first day. Approximately this amount of extra nitrogen was eliminated in the next 2 days. The intake of food was reduced during this period. This effect, plus the washing out of retained nitrogen, resulted temporarily in a very markedly negative nitrogen balance.

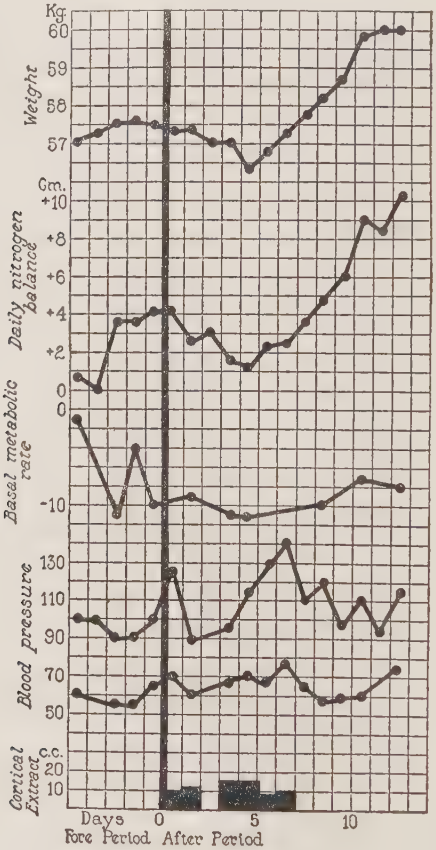

FIG. 15. EFFECT OF THE CORTICAL HORMONE ON THE NITROGEN BALANCE IN A CASE OF ADDISON'S DISEASE

The effect of treatment with the cortical hormone in increasing appetite and causing gain in weight has been stressed. This is well shown in the figures for intake of food in tables 16 and 17. The initial nitrogen balance in this group of patients apparently varied with the status of the weight curve at the onset of the experiment; it was positive in the 2 cases in which the patients were gaining weight and was in equilibrium or negative in the cases in which the weight was constant or falling. The majority of patients gain weight as a result of this form of treatment. This is accompanied by retention of nitrogen with a corresponding shift in the nitrogen balance. In the fourteenth case in our experience (table 17) there was a maximal daily retention of 10.09 grams of nitrogen. A normal concentration of blood urea at that time indicated that this positive balance was evidence of growth and not of urinary retention. At no time was there evidence of an abnormal or toxic destruction of protein.

The gain in weight of thirteen of the patients treated is indicated in the table. It must be remembered that some were in the hospital for short periods, whereas others remained for a period of a month or six weeks. The majority of the patients gained weight, and some did so in a very striking manner. Three gained 10 pounds (4.5 kgm.) or more in the course of a month. Associated with the gain in weight there is a corresponding shift in the nitrogen balance with nitrogen retention, as shown in figure 15.

Changes in weight (pounds)

CASE	DAYS UNDER TREATMENT	MINIMUM	ON DISMISSAL	GAIN
1		108	117	9
2	42	119	128	9
3	6	151	154	3
4	5	126	128	2
5	3	118	119	1
7	30	115	122	7
8	24	136	137	1
9	35	119	132	13
10	31	108	114	6
11	34	119	123	4
12	30	108	128	20
14	70	125	142	17
15	68	111	107	−4
17	63	113	120	7
18	9	123	123	
19	15	151	153	2

Renal function. It is recognized that renal insufficiency may be manifest during the critical periods and terminal stages of Addison's disease. It has been our clinical experience that an adequate intake of fluid is necessary in these cases, and that reduction in the latter, sufficient to permit a urinary output of less than 1 liter a day for 2 or 3 days, may be of serious consequence.

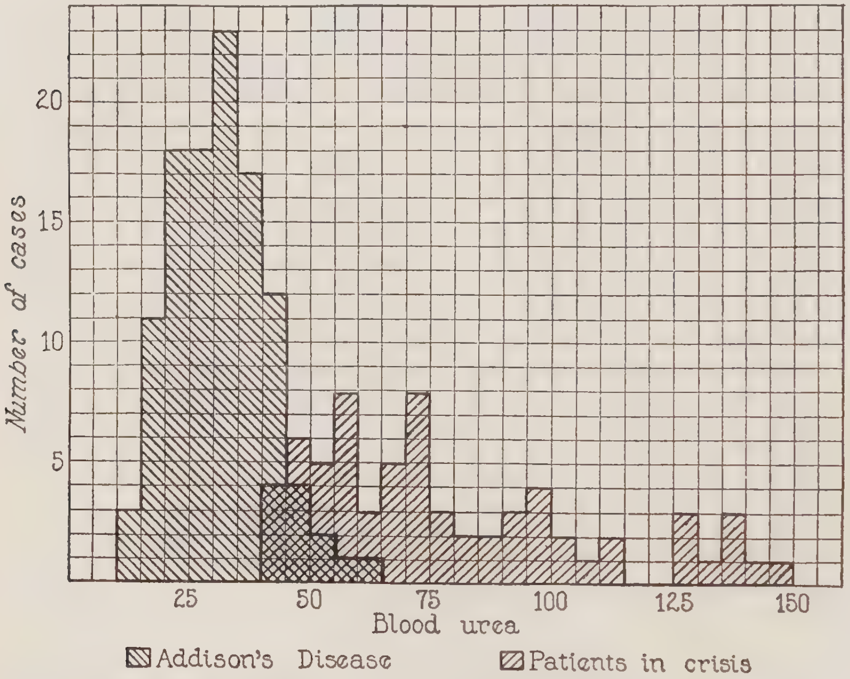

Fig. 16. Frequency of Distribution of the Concentration of Urea in the Blood in a Series of Cases of Addison's Disease

Cases in which the concentration of urea was 40 mgm. in each 100 cc. of blood, or more, and in which the patient was in crisis or was recovering from crisis, are marked.

Study of patients with Addison's disease who were in good clinical condition and whose intake of fluid was adequate, for the most part reveals normal values for blood urea and for excretion of phenolsulphonephthalein. The values for urea in each 100 cc. of blood were practically all less than 40 mgm. (fig. 16). Values of more than 50 mgm. with a maximal value of 154 mgm. were found only if patients were in crisis, vomiting, dehydrated, or in those who were in collapse or were recovering from this condition. An intensive study of renal function of a patient in crisis is not practicable, and our observations were limited. There is a suggestion, however, that the excretion of phenolsulphonephthalein is reduced at such times. The concentration of creatinin in the blood may be increased when the patient is in crisis; the maximal value observed was 4.8 mgm. in each 100 cc. The blood creatinin is a less sensitive index in these cases than is the blood urea and may still be within the normal limits when the latter is considerably elevated. The concentration of uric acid in the blood has been recommended as an index of renal insufficiency, but it did not depart from normal in this group of patients. Wakefield has pointed out the significance of an increase in the sulphates of the serum as an early sign of renal insufficiency. In Addison's disease he found the concentration of sulphates to be normal except during the critical periods and terminal stages of the disease. We are able to confirm this observation. The maximal value obtained was 13.5 mgm. in each 100 cc. Occasionally, the value for sulphates in the serum is slightly increased in a case in which the value for blood urea is normal and in which the patient is in fairly good clinical condition. This may well be an incidental observation, for it has been our experience that although there is retention of urea, creatinin and sulphates in the blood of a patient in crisis, and that a period of increased intake of fluid, with diuresis, will dispel this retention, the sulphates are the last to be eliminated and the slowest to return to normal. We have also observed 1 patient who died from Addison's disease, whose blood urea and sulphates were kept within a normal range by means of forced administration of fluids. These results we believe indicate that renal insufficiency in Addison's disease is a secondary manifestation dependent primarily on such factors as dehydration and circulatory collapse rather than being a direct manifestation of suprarenal insufficiency.

Other elements may enter into this response. Rowntree and Rosenow have both called attention to the delay in the excretion of water by these patients, and a change in the water balance may predispose to anhydremia. However, the fundamental relations we believe are those outlined.

c. Chemical condition of the blood

Some data on the chemical composition of the blood in Addison's disease are presented in table 18. The values for total solids, protein, calcium and potassium are increased and those for chlorides are decreased. Uncompensated acidosis, with changes in hydrogen-ion concentration, carbon dioxid tension, and carbon dioxid combining power in the blood of adrenalectomized animals also have been reported. We can confirm the presence of similar changes in clinical cases but again find that the severity of the disturbance varies with the stage of the disease. The concentration of serum proteins varies from 5.0 to 7.9 grams in each 100 cc. among patients who are in good condition. Values between 8.3 and 9.9 grams were found at the time of crisis. The values for calcium were normal in the cases of the first group, but they varied between 11.6 and 13.5 mgm. in each 100 cc. of blood in the cases in crisis. The values for potassium were similarly changed, but these changes were not very striking. The changes in the serum protein and calcium observed in cases of Addison's disease are characteristic of dehydration or anhydremia, with concentration

TABLE 18

The chemical composition of the blood of patients with Addison's disease

SERUM PROTEIN, GRAMS IN EACH 100 CC.		SERUM CALCIUM, MGM. IN EACH 100 CC.		SERUM POTASSIUM, MGM. IN EACH 100 CC.		SERUM INORGANIC SULPHATE, MGM. IN EACH 100 CC.		SERUM CHLORIDS, MGM. IN EACH 100 CC.		CARBON DIOXID COMBINING POWER, CC. IN EACH 100 CC. PLASMA		LACTIC ACID, MGM. IN EACH 100 CC.	BLOOD SUGAR, MGM. IN EACH 100 CC.
In chronicity	In crisis	In chronicity	In crisis	In chronicity	In crisis	In chronicity	In crisis*	In chronicity*	In crisis*	In chronicity*	In crisis*	In chronicity	In crisis
6.3	8.3	10.8	11.8	16.3	25.2	4.9	6.7	645	565	52	40	9.0	70
7.3	9.3	10.4	11.8	19.3	28.8	3.5	6.4	575	540	48	30	11.3	90
7.9	9.9	10.8	11.6	17.8	33.3	3.2	6.6	605	445	60	48	5.9	105
5.7	8.3	10.2	12.4	17.9		3.3	7.7	579	540	58	44	5.1	50
5.0		10.5	13.5	24.2		4.3	8.0	620	569	48	47	11.7	50
7.1		9.4		20.1		3.5	10.5	578	300	45	54	14.2	45
6.8		10.0		24.0		3.2	13.3	694	495	55	56		43
						4.1	7.8	651	535	52	35		58
							10.1	594	528	41	46		67
							11.7	519	536	68	36		85

* Ten cases taken at random; complete data not given.

of the blood. Direct evidence of anhydremia during crisis has also been presented by Rowntree and Brown, who found the blood volume and plasma volume reduced at such times, although normal values were the rule when the patient was in good condition. Changes in the blood which are consequent on dehydration and concentration must be considered secondary and not direct results of suprarenal insufficiency.

The values for serum chlorides may be low in Addison's disease, although not markedly so, for we find a variation of between 519 and 711 mgm. in each 100 cc. Certainly the values are not greatly different from the usually accepted figures for the normal. Lower values occur when the patient is in crisis, 455 to 569 mgm. Anorexia and vomiting are both pronounced at the latter time, and the combined effects of reduced intake of salt and loss of chlorides in the gastric content may well account for this reduction. We have previously pointed out that urinalysis did not indicate any great disturbance of the acid base balance of patients with Addison's disease. The carbon-dioxid combining power of the blood has varied between 41 and 68 volumes per cent of plasma in such cases. Some, but not all, patients who were in crisis gave evidence of acidosis. The range of variation of the carbon dioxid combining power in this group was between 30 and 50 volumes per cent. The retention of sulphates and of acids in consequence of renal insufficiency has been considered, and such retention may be a factor in producing acidosis. We have recently seen a patient in the terminal stages of Addison's disease to whom it was possible to administer sufficient fluids to insure adequate renal function and to maintain the blood urea and sulphates at normal levels. She was unable to take food, and after several days of virtual starvation the carbon dioxid combining power fell to 15 volumes per cent, while large amounts of acetone and diacetic acid appeared in the urine.

Considerable reduction in the degree of acidosis occurred in consequence of intravenous administration of glucose. The acidosis of this patient was due primarily to the ketosis of starvation. They suggest that although patients with Addison's disease as well as suprarenalectomized dogs show disturbances in the acid-base equilibrium, these changes apparently are secondary and are not to be considered as primary manifestations of suprarenal insufficiency. Cholesterol and the blood lipoids were also studied in a few cases, but the results were not greatly changed from normal.

A study of patients with Addison's disease disclosed that the fasting blood sugar, determined by the Folin-Wu method, varies between 70 and 130 mgm. in each 100 cc. with the value in the majority of the cases between 70 and 105 mgm., and the peak of the distribution curve between 85 and 90 mgm. Values below 70 mgm. were found only if patients were in crisis, and we have seen 12 cases in which the readings of blood sugar were between 45 and 70 mgm. This degree of hypoglycemia does not necessarily accompany the state of crisis. Symptoms suggestive of this condition have been observed by us, but are much confused with the preëxisting evidences of circulatory failure, coma and collapse. In such cases, gradual increase in the fasting blood sugar has been associated with recovery following treatment.

The slight reduction in the fasting blood sugar seen in cases of Addison's disease may be an expression of the anorexia and poor nutritional state which is characteristic of this disease. If, as suggested by others, it represents a characteristic and fundamental disturbance in carbohydrate metabolism, this must be of a special type unrelated to that seen in diabetes mellitus, for we have seen 2 cases, reported by Allen, in which the two diseases were coexistent.

The metabolism of lactic acid is closely related to that of carbohydrates. The lactic acid content of the blood of 4 patients with Addison's disease varied between 5.1 and 11.3 mgm. in each 100 cc. These are within the usual normal limits, although they come within the lower portion of the latter.

d. General summary of clinical results of use of the cortical hormone

Treatment has been given in The Mayo Clinic in 38 cases; these may be divided as follows: Addison's disease, 20 cases;[4] exophthalinic goiter, 3 cases; myxedema, 1 case; diabetes, 2 cases; neurasthenia and psychoneurosis, 5 cases; myasthenia gravis, 1 case; anorexia nervosa, 4 cases; myositis of undetermined origin, 1 case; postural hypotension, 1 case.

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[4] Since this was written 12 additional cases have been treated at the Mayo Clinic with extract furnished by Dr. Oliver Kamm and made according to the technique previously outlined.

Five of the patients with Addison's disease died, three at home and two while under treatment at the Clinic. Four of this group of five responded admirably to treatment; on three different occasions one of them was brought from collapse almost to apparent health. Two of the three who died at home did not have further treatment with the cortical hormone; both died several weeks after their return home. The third patient of the three had received only 40 cc. of the cortical hormone when under treatment at the clinic in July, 1930. He returned to his home in Montana to continue treatment by the Muirhead regimen. In April, 1931, he was reported to be in crisis by his home physician. Some of the cortical hormone was forwarded, and three days later 20 cc. was injected, but the patient was moribund and relief could not have been expected.

One of the two patients who died while under treatment at the Clinic was admitted in a state of collapse. Our total supply of cortical hormone was 40 cc. Three other patients were waiting treatment, one of whom was rather seriously ill, but treatment had been postponed for five days. The patient in collapse was given 20 cc. of the hormone, all that could be spared. There was slight improvement but death ensued within a few hours. The other patient who died while under treatment also was admitted in a serious condition and was given a course of the cortical hormone to which he responded admirably. After a week or ten days, because of lack of hormone, another form of specific treatment was tried, but the patient went into collapse. Sixty cubic centimeters of the cortical hormone was then given, but the patient died.

Of the three patients who died while receiving treatment elsewhere, only one patient received what we considered an adequate amount of the cortical hormone. It might be argued that if an adequate supply of the hormone had been available death might not have occurred in these cases, but it is possible that patients *in extremis* are beyond aid, and that even if the supply of hormone had been adequate the results would have been the same.

In the 20 cases of Addison's disease the immediate results were excellent in 15. As has been mentioned, five patients died. Two patients are still under treatment. Two patients have been observed over a long period and have been given what, in our opinion, constitutes an adequate amount of the hormone, but without satisfactory response. The first of these is a patient from Colorado who failed to respond in the usual favorable manner. Since his return home the hormone has been forwarded, we believe, in adequate amounts, but there has been little, if any, improvement, and loss of weight and strength has been continuous. Results with the second patient suggested the possibility that the cortical hormone might be inadequate in the treatment of Addison's disease. The patient's condition had been diagnosed elsewhere as Addison's disease. Immediately on her arrival at the Clinic the cortical hormone was given. The disease was typical in every respect. The patient was apprehensive and nervous. Several untoward symptoms developed following the administration of the hormone, consisting of increased nervousness, tremor, chilly sensations and prostration. In the beginning this was ascribed to the hormone, which was not prepared by Swingle and Pfiffner. However, when the preparation forwarded by Swingle and Pfiffner was given the same symptoms developed, proving that the preparation was not responsible. This patient had a metabolic rate of −33 per cent, which increased under treatment to −13 per cent. Subsequently, small quantities of thyroid extract were given and the reaction of the patient indicated increased sensitiveness to this extract. She did not gain materially in weight or strength. Blood-pressure increased slightly and some decolorization was noted. The patient died while undergoing treatment. Almost complete atrophy of both suprarenal glands was present.

Treatment in diseases other than Addison's. Subjectively, two or three patients with exophthalmic goiter felt considerably better. Objectively, they showed evidence of less stimulation and some increase in strength, with slowing of the pulse and decrease in the basal metabolic rate from 60 to 30 per cent in one case and from 55 to 28 per cent in the other. Practically, the results cannot be considered of consequence, since both patients had been given compound solution of iodine and had been operated on. Scientifically, however, the results were of interest and make us feel that the suggestion of Marine as to the participation of the suprarenal gland in exophthalmic goiter should be thoroughly investigated.

In two of four cases of anorexia nervosa there was slight improvement; appetite developed in one case and an increased ability to take food developed in the other. In five miscellaneous cases of neurasthenia and psychoneurosis results were practically negative. One of these five patients, a physician, whose body was of the Lorraine type of infantilism with osteoporosis, had been given an indeterminate diagnosis, but he believed himself to be suffering from Addison's disease. This was not supported, in our opinion, by the history or the physical condition. His case was not included as one of Addison's disease, but the patient insisted that he was greatly benefitted by the cortical hormone. None of the other diseases was affected favorably by the cortical hormone.

Since the summer of 1930 Doctors Harrop and Weinstein have been engaged in an intensive clinical study of the use of the cortical hormone in Addison's disease. Their findings have not as yet been reported *in extenso*. A brief discussion of their results obtained on eight cases has appeared in the Journal of the American Medical Association, 1932, 98, 1525.

e. Conclusion

It is important to bear in mind that the clinical results here reported were obtained by use of cortical extracts of relatively low potency. The preparations employed in all of this work represented our earlier material which assayed but 4 to 10 D.U. per cubic centimeter. Our recent extracts made from whole glands assay 40 to 80 D.U. units per cubic centimeter and hence are many times more potent than the early dissected cortex material used for clinical tests. The matter of dosage with this more potent extract and the daily requirements of Addison's disease patients have not been worked out as yet. This is a clinical problem which yet remains to be solved. Judging from our assay experiments on dogs, the quantities of extract heretofore employed in the clinic on the patients are far greater than is necessary when using this more potent preparation. The latter type of extract has been clinically tested upon three severe cases of Addison's disease by Dr. Carl Greene with excellent results, but the detailed study is not yet ready for publication.

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SALT AND WATER METABOLISM IN NEPHRITIS

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Nephritis has played somewhat the same rôle in the advancement of knowledge concerning the metabolism of water and salts that diabetes has in the intermediary metabolism of the foodstuffs, carbohydrate, protein and fat. It has afforded magnificent ready-made experiments for the clinician to observe, has stimulated him to more modest experiments and to imitate nature by attempting to reproduce the disease in animals. Out of the mass of observation and experiment that has resulted a few important principles appear to have been established beyond reasonable doubt. The disease nephritis, with all its diverse manifestations, has not been successfully reproduced in experimental animals. A large proportion of the clinical symptoms and functional disturbances of the disease can not be produced by and probably are not directly referable to renal injury. It would seem to follow from this that nephritis must be considered as a generalized disease or one that gives rise secondarily to disturbances of organs or organ systems other than the kidneys. Most of the functional disturbances which have, from time to time, been hailed as characteristic or pathognomonic of the disease have, on further investigation, proved to be merely associated with some symptom of the disease, and, in this association, to hold the position of result rather than cause. Finally, although the responses of the nephritic patient are disorganized in a quantitative sense, they are qualitatively similar to those of the normal animal. The nephritic exhibits the usual tendency to maintain an internal environment that permits life to proceed in an orderly and efficient manner; but, under the compulsion of his incapacity, is forced to innumerable compromises.

It is idle, therefore, to study or discuss the disease except in relation to physiology and to other abnormal states which are accompanied by analogous symptoms. Obviously it is beyond the scope of this review to cover in a comprehensive critical manner the whole physiological background involved. The setting must be assumed and, in the assumption, betray the predilections of the author.

To discuss the subject from the standpoint of the physiological mechanism of urine formation, even if this were more certainly known, is impracticable, as nephritis is not simply a renal injury. Nor is our knowledge of physical chemistry yet sufficiently advanced to permit more than a limited and indefinite application of its quantitative laws to equilibria in such complex systems as the human body. Of necessity, therefore, the treatment must be pragmatic and somewhat haphazard, following sometimes salient symptom complexes, at other times abnormalities of chemistry and metabolism which can not yet be connected with any more obvious clinical manifestations.

In the historical introduction to "Die Bright'sche Nierenkrankheit und deren Behandlung" 1851, Frerichs (120) points out that the relationships between kidney disease, urinary changes and edema had been, to some extent, recognized by Galen, Aetius, Avicenna, Schenck, Bonet, Lieutaud and Morgagni, the connection between albuminuria and hydrops by Cortugno, Cruikshank, Wells and Blackall (37) before the nineteenth century. However, the descriptions of these writers were so vague and their deductions denoted so little appreciation of the significance of their observations that the world has properly ascribed priority in the discovery of nephritis and its manifestations to Bright (67, 68, 69, 70), whose studies appeared in his medical case reports and the Guy's Hospital reports from 1827 to 1843. These papers are models of astute observation, objective description and brilliant deduction. With such modesty are they presented, however, that many of their contributions have been overlooked and ascribed to others by whom they have been rediscovered subsequently. Besides the clinical and pathological manifestations of the disease which bears his name, Bright recognized or, with his associates, Bostock, Barlow and Rees, discovered many of its attendant chemical and metabolic abnormalities. Chief among these are the proteinuria and the origin of urine protein from serum proteins, the reduction of the latter and its association with edema, the accumulation of urea in the blood in advanced stages of the disease, the large volume and low specific gravity of the urine in chronic cases.

Perhaps the most conspicuous functional disturbances in nephritis are those connected with the excretion of water and with the distribution of the same component within the organism. The most obvious of these disturbances are: hyposthenuria (inability to concentrate solutes in the urine) and its associate, polyuria, on the one hand; edema and oliguria on the other.

HYPOSTHENURIA

The low specific gravity of the urine of patients with hydrops and proteinuria was mentioned by Blackall (37), who suggested that it was due to deficiency of urea in the urine. It received increasing emphasis by Bright (67, 68, 69) as his studies progressed and was recognized by his associates, Bostock (68), Barlow and Rees (69, 263, 264) and his immediate successors, Christison (83), Rayer (262), etc., as a symptom of importance indicating inability to concentrate solutes, especially urea. The same observers noted the associated polyuria and rightly inferred that this served to compensate for the loss of concentrating powers, preventing or delaying the accumulation of excretory products in the blood and tissues. That dilute urine was more characteristic of the later, more chronic and non-edematous stages of the disease and that the specific gravity might be high in the acute and subacute stages, especially when there was profuse albuminuria and edema, is clearly stated by both Bright and Christison.

Bradford (62), Pässler and Heinecke (240), Mark (205) and others, in experimental studies on the effects of subtotal extirpation of the kidneys, have found that hyposthenuria is the only invariable functional disturbance produced by loss of kidney substance. The ability to excrete water is retained up to the premortal period. In fact, polyuria is regularly associated with hyposthenuria. In advanced destructive lesions of the kidney and in clinical and experimental partial ureteral obstruction (161) hyposthenuria is also regularly encountered. For this reason it is considered by v. Korányi (322), Volhard (320), Fishberg (115), Addis (6, 7) and others as the only sign of true renal insufficiency.[1]

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[1] The word "insufficiency" was, perhaps, unfortunately chosen in this connection, because it has not its usual functional significance. Perhaps "deficiency" would be more exactly descriptive. The excretory function of the kidney may be insufficient, in the

Hyposthenuria might be due not to inability to concentrate solutes, but, as it is in diabetes insipidus, to an obligatory polyuria. In nephritis, however, inability to concentrate appears to be the primary factor. Restriction of fluids, while reducing polyuria, does not increase the specific gravity of the urine proportionately, but causes the usual urinary solutes to accumulate in the blood. The polyuria and the thirst and craving for fluids which accompany it are aggravated by increased protein intake or nitrogen metabolism (62), and can be diminished or abolished by measures which reduce the necessity for the excretion of solutes. The polyuria, therefore, seems to be merely a compensatory expedient by which the organism is enabled to excrete the necessary quantity of waste products in high dilution.

v. Koranyi (322) with his associates, Kövesi and Roth-Schulz (169), were apparently the first to appreciate the full significance of hyposthenuria as a measure of renal function and to investigate its nature extensively. For this purpose he employed freezing point determinations because the depression of the freezing point, Δ, is a measure of the total concentration of chemical components in a solution. As the result of his studies he came to the conclusion that as renal function decreases the molecular concentration of the urine first diminishes because of loss of concentrating powers; later the diluting power also fails, the molecular concentration approximating more and more that of the serum,—i.e., becoming less and less variable, approaching as its limit − 0.56°C. Similar observations have shown a corresponding tendency for the specific gravity to approach 1.010 to 1.012. v. Koranyi offered as an explanation for this phenomenon the fact that with failing function the capacity of the kidney to perform the work entailed in the processes of concentrating and diluting solutes withdrawn from the serum, progressively diminished. This theory implies an interdependence of solutes, which has been contested by Frerichs (120), Ambard (15), Widal (331), Schlayer (284) and others,

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sense that it is inadequate for the needs of the body, in the absence of hyposthenuria. Evident examples are found in persistent vomiting, severe diarrhea and edematous stages of nephritis, pneumonia, states in which, as a result of extrarenal disturbances, there is not sufficient fluid available for urine formation to permit the excretion of the waste products of metabolism rapidly enough to prevent some accumulation in the body, either because of increased production of waste products, reduction of available water, or both.

and certainly has not been established beyond doubt for either the normal or the diseased kidney. Pepper (243) has presented statistical data from patients with nephritis indicating that the concentration of urea + Cl (in terms of molecular equivalents) is more constant than is the concentration of either urea or Cl.

v. Korányi (322) favored the Haidenhain theory of urine formation rather than the more mechanistic theory of Bowman and Ludwig. More in keeping with the latter is the hypothesis of Fishberg (114, 115, 116) that the ability of the kidney to elaborate from a protein-free filtrate of serum urine in which constituents appear in far different concentrations diminishes progressively as renal function decreases, and that in this process all solutes are equally affected, the urine finally approaching in composition a simple filtrate of blood plasma with a specific gravity of 1.010 to 1.012. The tendency for this phenomenon to evidence itself in some solutes more than in others he ascribes to the differences in the demands for concentration of the various solutes. For example, under ordinary circumstances, the concentration of urea in the urine may be 1000 times as great as that in the serum, while the concentration of chloride in the urine is only 2 or 3 times as great as that in serum. In terms of the theory of Bowman and Ludwig, as the functional capacity of the kidney diminishes the glomerular filtrate from the serum undergoes less and less transformation in its progress through the tubules. Mayrs (214) believes this is due to failure of the tubules, because of injury or destruction, to reabsorb water. Fremont-Smith (119) has advanced a more elaborate explanation. He suggests that when a large proportion of the glomeruli are destroyed, the remainder are forced to work harder or more continuously. With their capillaries wide open they permit the passage of an unusually large filtrate. This, in turn, traverses relatively few tubules at such a rapid rate that there is little reabsorption. Although both of these concepts, and especially the latter, offer ingeniously satisfactory explanations of the phenomenon of hyposthenuria, they have not been supported by objective evidence acceptable even to those who have a predilection for the more mechanistic theory of urine formation.

How universally the urine solutes are affected by hyposthenuria can not yet be considered as settled, nor can it be settled by the usual methods of investigation, which involve analysis of urine only. Certainly it can be established only for substances the concentration of which in the body is controlled by the kidney and which are excreted by the kidney without obvious chemical transformation. It must also be proven that under the conditions of an experiment they are offered to the kidney for secretion in adequate amounts, that is, the stimulus to the kidney to concentrate the given substance must be great. As a corollary it follows that if, for lack of water, a given substance is not excreted, it should accumulate in excess in the blood. (Such accumulation will, theoretically, lead to increased urinary concentration.) Urea is the only solute for which all these postulates can be said to have been satisfied with certainty. Among inorganic components claims have been made for chlorides (97, 169, 224), phosphates (98, 169, 216), acids and alkalis (182, 275). The validity of these claims will be analyzed in subsequent sections in which these components are discussed separately.

The ability of the kidney to dilute the urine, that is, to excrete a large volume of water, has been found, both experimentally and clinically, to be among the last of its functions retained. Nevertheless there seems to be little doubt that it does finally diminish, first relatively (that is, in proportion to the solutes excreted), finally absolutely. During the earlier stage the renal insufficiency may be considered as compensated in the sense that by the excretion of a large volume of dilute urine the excretory needs of the body are fulfilled; in the later stage the urinary volume can not be increased sufficiently to compensate for the hyposthenuria. It is in the stage of decompensation that retention of waste products in the blood occurs. Although loss of diluting power may develop as the result of renal disease *per se*, in actual point of fact its appearance in clinical conditions is far more often referable to extrarenal factors, especially heart failure or the diversion of water for the formation of edema. Chronic passive congestion impairs the ability of the kidneys to excrete water without diminishing their capacity to concentrate solutes (or at least urea). The effect of the edematous tendency in nephritis appears to be similar. The combination of heart failure or the edematous tendency with renal insufficiency will, therefore, result in loss of both diluting and concentrating powers, the picture of renal decompensation, with urinary specific gravity fixed in the neighborhood of 1.012 (224). It is probably such combinations which led v. Korânyi (169, 322) to conclude that the molecular concentration of the urine is more invariable in chronic parenchymatous than in chronic interstitial forms of the disease, an opinion at variance with that of most observers. Loss of diluting power may be considered as a measure of the inability of the kidney to perform its function, whether it results from renal or extrarenal factors. The etiological distinction is, however, important, if it is to be used as a criterion of the extent of renal disease.

It is impossible to discuss the innumerable procedures which have been proposed for the determination of the presence and degree of hyposthenuria; it is, however, possible to mention and to criticize briefly the principles on which they depend. All ultimately aim to measure the variability of urinary concentration in terms of one of its functions: freezing point depression, specific gravity, volume, or some individual solute. Because of the technical difficulties involved, freezing point determinations have, in general, given way to the simpler specific gravity methods. Because ordinary conditions of life do not necessarily compel the kidney to concentrate urine and do not insure variability in concentration, Schlayer (147, 284), Mosenthal (224) and others have proposed standard dietary regimes on which patients are placed during the tests. The objections to such procedures is that they do not establish sufficiently extreme conditions to compel the kidney either to concentrate or to dilute the urine greatly, especially if the subject has subsisted during the preceding period on a diet which has been limited in salt, nitrogen or water (197). The Schlayer method is, perhaps, the better of the two mentioned because it includes a preliminary period of two or three days on a standard dietary. This, however, makes the procedure complicated and time consuming. Volhard (320) determines the specific gravity of the urine at intervals during the day after the subject has taken a single large dose of water in the morning. The earlier specimens show the diluting power of the kidney, later ones its ability to concentrate. The test, as Addis (7) has pointed out, insures dilution more certainly than it does concentration. The latter is, however, from the standpoint of renal function, the more important (4). For this reason Addis (6, 7) and others have proposed the use of measures which stimulate the kidney to exert its concentrating powers to the utmost. They study the urine specific gravity after the subject has been deprived of fluids for twelve to twenty-four hours. In general these tests appear to give the most consistently reliable information. However, as Pratt (257) and others (178) have shown, they may also fail of their purpose if the subject has previously received excessive quantities of fluid. Determination of the relative volumes of urine excreted during the day and the night has proved so unreliable that it has been almost abandoned. Lebermann (183) attempted to substitute for fluid restriction the injection of pituitary extracts, but secured irregular results in both normal and nephritic subjects.

Hyposthenuria is encountered in all conditions in which there is massive reduction of renal parenchyma; but is not demonstrable by ordinary methods unless more than half the kidney substance has been destroyed (62, 205, 240). It occurs in surgical diseases of the kidney, polycystic kidneys, partial urinary obstruction (224). It is more common in those types or stages of nephritis in which the substance of the kidney has been destroyed and its architecture altered by scarring (arteriosclerotic and chronic glomerular nephritis) than in the degenerative or tubular (nephrotic) forms or stages (224, 226, 84).[2] Although loss of concentrating powers and compensatory polyuria are regularly found in conditions associated with massive kidney destruction, they are observed in certain other diseases in which no renal pathology can be demonstrated. Among such conditions are diabetes insipidus, severe anemia (224) and advanced liver disease (8). It is also impossible to secure concentration in edematous cases during the course of diuresis, because restriction of fluids does not reduce the urine volume (322). Failure to dilute the urine is, for reasons given above, even less pathognomonic of renal disease than is hyposthenuria. Loss of diluting powers, presumably due to extrarenal causes, is found in all subjects with heart failure, existing edema or the tendency to edema formation. Failure to dilute from purely renal causes is a sign of the most advanced renal destruction and is found in the late stages of the same conditions that cause hyposthenuria (6, 116, 224). In the degenerative forms or stages of nephritis, when edema and the edematous tendency are in abeyance, both concentrating and diluting powers are usually normal (84, 224).

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[2] A certain caution must be exercised in the interpretation of the specific gravity of urine which contains albumin. The latter exerts a definite influence upon specific gravity; but is not a normal urinary constituent. If there is any considerable quantity of protein this must be removed before the specific gravity is determined or else a correction factor must be employed. It has been estimated by Lashmet and Newburgh (178) that 1 per cent of protein raises the specific gravity of the urine by about 0.004. Pepper (243) states that a 1 per cent solution of protein at 15°C. has a specific gravity of 1.0024. According to the data of Moore and Van Slyke (223), in plasma each increase of 1 per cent of protein raises the specific gravity by 0.0029.

EDEMA

Bright (67, 68, 70) and his associates (263, 264) appear to have been the first to suggest that the hydropic tendency in nephritis was connected with "thinning" of the blood, which they detected by determinations of serum specific gravity and correctly referred to reduction of serum proteins, the result of leakage of proteins into the urine. Although Bright mentions the increased water content of the serum he seems to recognize that this is due to loss of protein rather than accession of water. The relation of hypoproteinemia to transudation is more explicitly stated by Christison (83) and Sabatier (269). The latter says: "La sérum du sang se trouve donc appauvri d'albumine; il devient alors plus fluide, plus ténu, et par cela même il peut pénétrer plus facilement les parois des capillaries artériels. Si, comme il est permis de l'admettre, d'après cette modification du sang, l'absorption veineuse est moins active, on concevra des- lors comment se forment, en pareils cas, les épanchemens dans les cavités séreuses et les infiltrations du tissu cellulaire." Solon (297), a little later, while adhering to the same general theory, and citing in its support experiments of Magendie, introduces the additional idea of an increase of blood volume, which he ascribes to the interruption of the normal excretion of water by skin and lungs. Although the theoretical significance of the hypoproteinemia continued to be recognized (120, 285, 302) its importance in the production of nephritic edema is less appreciated from this time on. Rayer (262) relieved the serum protein deficiency of all responsibility because it did not develop in acute nephritis until after the edema had appeared and because edema did not occur in "tropical endemic hematuria." The comparatively late appearance of the serum protein deficit in acute nephritis has proved an insurmountable objection to many observers since Rayer. The conception of hydremic plethora, advanced by Solon, became more and more prevalent and led to the experiments of Cohnheim and Lichtheim (86). Because they were unable to produce edema in animals by injections of fluid of enormous magnitude or by removing blood and replacing it with saline, they concluded that the permeability of the vessels must be increased in nephritis. These experiments, which have been repeated by Boycott (57), Chisolm (82) and others, seemed to dispose effectually of the hydremic plethora theory. They were interpreted by many, also, as evidence against the causal relation between hypoproteinemia and edema.

It is interesting to note that in spite of the emphasis placed on the renal lesions by all these earlier observers, edema was almost universally attributed to extrarenal factors. The persistence of the hydremic plethora theory despite Cohnheim, and the demonstration by Strauss (304), Widal (329), v. Korányi (322), Kövesi and Roth-Schulz (169) and others of the effect of sodium chloride in promoting edema, completely altered the views of the clinical world. Not only the possible rôle of hypoproteinemia in the production of hydrops, but even the very existence of a serum protein deficit in nephritis was neglected, or else the deficiency was ascribed to hydremia. Nephritic edema was almost unquestioningly attributed to failure of the renal excretory function and controversy centered entirely about questions of priority: whether it was water, sodium chloride or one of the ions of sodium chloride which was primarily retained. This work and controversy will be discussed later; for the moment it may be considered an interruption of history

Cohnheim (86) had been unable by the experimental production of hydremic plethora to cause edema in animals. Others had concluded that the serum protein reduction of nephritis was not due to increased blood volume because it was not attended by a comparable diminution of red blood cells or hemoglobin. Within recent years measurements of the blood volume have been made with dye methods by Linder, Lundsgaard, Stillman and Van Slyke (190), Darrow (87, 89) and Brown and Rowntree (72, 73) and with carbon monoxide methods by Waterfield (325). All have agreed that edema of nephritis is not accompanied by hydremic plethora; in fact Darrow and Waterfield found the blood volume in most cases below rather than above normal.

In 1912 Epstein (108) called attention to the serum protein deficit of patients with nephrosis and the rôle which Starling's (299) physiological studies had shown that this must play in the production of edema. Starling had advanced the theory that the exchange of fluid between the blood in the capillaries and the tissues was controlled by two opposing forces: (*a*) hydrostatic pressure (capillary blood pressure), tending to force fluid out of the capillaries; (*b*) the osmotic pressure of the plasma proteins, to which the capillary walls were ordinarily impermeable, tending to draw fluid into the blood stream. Experimental support has been given to the theory by Krogh (173) and others. Schade and Claussen (277) and Govaerts (128) extended its application to the general problem of edema, not only in nephritis, but in other diseases. They determined the osmotic pressure of the colloids (*oncotic pressure*) in various diseases and compared it with the concentration of proteins. The correlation between reduction of oncotic pressure and edema in nephritis was demonstrated. Govaerts estimated the osmotic pressure of the serum protein fractions and found that of albumin far less than that of globulin, as one would expect from their relative molecular weights.

Although many observers still refuse to accept the application of Starling's theory to edema production, evidence in its support has steadily increased. Before reviewing this evidence it may be well to state briefly the practical application of the theory to the internal fluid exchange. Ordinarily at the arterial end of the capillaries the blood pressure exceeds the oncotic pressure (the osmotic pressure of the colloids, chiefly proteins, to which the capillary walls are impermeable), therefore fluid is forced from the capillaries into the lymph spaces and interstitial spaces of the tissues. As the blood progresses towards the venous end of the capillaries the blood pressure diminishes steadily, finally falling below the oncotic pressure. At this point fluid returns to the vessels. If the oncotic pressure is abnormally low, which happens when the proteins are reduced, the tendency for fluid to escape from the vessels will be increased. Reduction of the albumin fraction of the proteins is far more important than reduction of globulin because the osmotic pressure of albumin is 3 or 4 times as great as that of globulin (128). If the hydrostatic pressure is increased by any impediment to the venous return, as it is in heart failure, transudation from the capillaries is augmented. Finally, passage of the fluid from the blood to tissues is accelerated if the vessel walls become permeable to proteins, permitting the latter to escape into the interstitial spaces. Although the loss of protein may not be great enough to lower seriously the serum proteins, it will diminish the effective oncotic pressure, because this is dependent upon the difference in protein concentration on the two sides of the capillary membrane. Such increased permeability explains the occurrence of edemas due to inflammation and injury. It is obvious that, although low oncotic pressure will always conduce to edema, correlation between serum protein concentration and edema will be secured only in those conditions in which the capillary blood pressure and the permeability of the capillaries remain normal.

Edema fluids and transudates from patients with nephrotic types of renal disease (nephrosis, amyloid nephrosis and chronic nephrotic glomerulonephritis) have been found by numerous observers (30, 110, 111, 244, 278) to contain minimal amounts of protein, less than 0.5 per cent. This is the best evidence that the permeability of the general capillary system is not disturbed. Moreover, hypertension, arterial disease and cardiac failure are rare in these conditions, so that the complicating factor of circulatory stasis is seldom encountered. It is in these diseases, then, among renal conditions, that one may expect to find close correlation between serum protein or serum albumin concentration and edema. That the correlation is extremely close has been demonstrated in large series of cases by Moore and Van Slyke (223) and by Peters, Bruckman, Eisenman, Hald and Wakeman (245).

In the types of nephritis associated with hypertension and arterial disease, edema, when it occurs, is usually referable to heart failure and may, therefore, appear at any level of serum proteins. Although it is not uncommon in these conditions to find edema when the serum proteins are high; the converse, absence of edema when the proteins are greatly reduced, is not encountered (247).

Since the time of Bright one of the chief objections to theories connecting the serum protein deficit with nephritic edema has been the well recognized fact that in acute nephritis dropsy may appear long before the proteins have fallen. This objection has been largely removed since Beckmann (30) and others (244) have demonstrated that the transudates in the early stages of acute nephritis contain protein in relatively high concentration. This is an indication that the permeability of the general capillaries is disturbed and is quite in keeping with the other evidences of vascular damage which regularly accompany the condition: hypertension, hemorrhages into the retina and other organs, etc. If edema persists after these manifestations of general vascular damage have subsided, evidences of either cardiac failure or reduction of serum proteins can be demonstrated (246). It may be well to emphasize again that ablation of renal substance does not give rise to dropsy. Injury of the kidney by such poisons as uranium may give rise to transudative phenomena; but it has been clearly proven that uranium injures the capillaries throughout the body and that the transudates produced under its influence contain protein in high concentration (128a).

In famine edemas (153, 279, 281, 282, 328) and the edemas that develop in patients with wasting diseases (30, 74, 167) serum albumin deficiency is regularly found. By means of low protein diets edema (121, 167) with hypoproteinemia has been produced. Finally, Leiter (184), Barker and Kirk (27), Shelburne and Egloff (294), Darrow (88) and Weech (328) have shown that edema appears uniformly in dogs when, by plasmaphaeresis, the concentration of proteins in the serum is reduced beyond a certain critical level.

There can be no doubt, from these experiments, that the reduction of serum proteins in patients with so-called "renal edema" (nephrotic types of nephritis) is great enough to cause dropsy without the intervention of any other factor. Furthermore, when edema occurs in nephritis without serious serum protein deficiency, evidence of extrarenal influences, increased permeability of capillaries or heart failure, can usually be demonstrated. If the subject is approached from this point of view the problem of nephritic edema seems to be quite satisfactorily settled without recourse to any explanations that involve impermeability of the kidneys to either salt or water.

Theories of edema have been advanced which involve such hypothetical conceptions as alterations of the nature or state of tissue colloids, disorganization of cellular membranes, changes in the reaction of tissue cells, hormonal or nervous influences, and abnormal distribution of electrolytes and water between blood and tissues or interstitial fluids. For discussions of these theories the reader is referred to articles dealing more exclusively with the subject of edema by Fisher (113), Loeb (191), Elwyn (105), Siebeck (295), Veil (317), Volhard (320) and others (12, 18, 19, 29, 30, 107, 226, 234, 1). It is characteristic of most of these concepts that they deny the operation of simple physico-chemical forces in the production of dropsy, ascribe to endothelial membranes highly selective permeability or secretory activities, and frequently imply that edema affects cells or that the various media in the body are not in osmotic equilibrium. Concerning the situation of edema fluids (whether intercellular or intracellular) it is impossible to secure exact information by chemical analysis, because tissue cells can not be completely isolated from the intercellular fluids. Inferential evidence, however, indicates that edema fluids are extracellular. Microscopic examination of tissues from dropsical patients reveals no appreciable general swelling of glandular or muscular cells, but distention of areolar tissues, separation of parenchymatous cells, and accumulation in serous cavities. The only cells which can be isolated for chemical analysis are the red blood cells. That the proportion of water and electrolytes to colloids in these cells is undisturbed in nephritis was first pointed out by Carl Schmidt (285) whose findings have been repeatedly corroborated by subsequent observers. The ratio, hemoglobin (expressed in volumes per cent of oxygen capacity) : volume of red blood cells (expressed in per cent), has been found by Eisenman (103) to be of the same order of magnitude in patients with renal edema as in normals. Shifting of edema under the influence of gravity and drainage by simple puncture of the skin would be inconceivable, were the fluid situated within the cells. Finally, the inorganic elements retained during edema formation and excreted during its resolution are those which are considered characteristic of the extracellular fluids: sodium salts, not potassium salts, which are the chief components of the tissue cells (123).

The most exact quantitative comparisons of transudates with serum are compatible with the hypothesis that such transudates, whether they occur as the result of venous stasis in heart disease, portal obstruction in hepatic cirrhosis, or in nephrotic forms of renal disease, are in osmotic equilibrium with the blood plasma and correspond to a more or less protein-free filtrate of the latter (126, 133, 144, 194, 229). The differences in electrolyte distribution are referable to the different protein contents of the two fluids and are maintained *in vitro* when an artificial membrane impervious to protein is substituted for the endothelial membrane which separated them in life (133, 194). Hypotheses involving intracellular edema, abnormal distribution of electrolytes or secretory activity of capillary endothelium appear not only unnecessary, but at odds with the best available analytical data.

THE RÔLE OF ELECTROLYTES AND WATER IN THE PRODUCTION OF EDEMA

At first sight the Starling theory of the production of edema with complete exoneration of the kidneys appears to neglect entirely the accumulated evidence demonstrating the dependence of edema upon salt and water metabolism. It was, in fact, the recognition of the influence of salt on retention of fluid that led v. Korányi (322), Strauss (304, 305), Widal (329, 330, 331) and others to the conclusion that the cause of dropsy lay in the impermeability of the kidneys for chlorides, a conception which still has many adherents. v. Korányi, Strauss and Widal demonstrated that restriction of sodium chloride in the diet exerted a diuretic effect in nephritis, whereas added salt furthered retention of fluid. Subsequently it has been shown that other sodium salts may have a similar hydropigenous effect (40, 41, 164, 199), while potassium salts of the same acids (41, 42, 97, 146, 164, 187) do not. About such observations have accumulated volumes of conjecture and controversy concerning not only the rôle of renal permeability in connection with edema, but also the relative influence or priority of water and the ions Na^+ and Cl^- in its production. Before attempting to analyze this literature it may be well to turn from nephritis for a moment to the consideration of normal salt and water metabolism and analogous dropsical conditions.

The predominant base of blood plasma and interstitial fluids is sodium, that of the cell potassium.[3] For their supply of sodium animals are dependent almost entirely on sodium chloride which may be added to food. Foods themselves contain almost entirely potassium salts. It is, therefore, relatively easy by restricting radically the intake of sodium and chloride in the diet to remove in great part the inorganic elements which are most essential for the maintenance of the normal constitution of the extracellular fluids. Under these circumstances, if the electrolyte pattern and the osmotic pressure of these fluids is to be maintained, the sodium salts must be replaced by salts of some other bases, the excretion of sodium must cease or an amount of water equivalent to the sodium which escapes must be withdrawn from the interstitial fluids and excreted by the kidneys. Apparently it is the last reaction which takes place. The electrolyte patterns and qualitative differences in the internal environment, which are presumably due to selective permeability of the cell membranes and connected with the inherent differentiation of cells, appear to be maintained. The conservative response of the kidneys is not perfect; some sodium and chloride continue to be excreted and with them an equivalent amount of water. The subject loses a certain amount of weight, apparently at the expense of the interstitial fluids, in order to maintain unchanged the electrolyte composition and osmotic pressure relations in the body (15, 76, 85). Large volumes of water, given without an equivalent quantity of salt, are rapidly excreted. With the diuresis thus produced a certain amount of sodium chloride is swept out of the body. This is eventually succeeded by the elimination of an equivalent amount of water, unless salt is given to replace that which was wasted. The ultimate result of such a water diuresis, then, is the sweeping out of a certain amount of endogenous salt and water; the subject loses weight at the expense again of the interstitial fluids (76, 85). Apparently a comparable reaction follows the administration of a single large dose of salt. A certain amount of body water is withdrawn to facilitate the excretion of the salt and subsequently extra sodium chloride equivalent to the fluid thus lost is eliminated.

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[3] Of course the composition of normal interstitial and serous cavity fluids can only be inferred, because it is impossible to secure material for analysis. Normal spinal fluid and non-inflammatory transudates of all kinds are characterized by high sodium, low potassium content. The same is true of the secretions of the gastro-intestinal tract. Simple dehydrating measures are attended by loss chiefly of sodium; while processes associated

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with cellular destruction increase the excretion of potassium (125, 270). The best available analyses of muscle and other highly cellular tissues reveal no more sodium than one could expect if this element were contributed entirely by the inseparable interstitial substance included in the analysis. Human red blood cells contain only very small amounts of sodium.

If an isotonic sodium chloride solution is given, or if both salt and water are given in large quantities, the diuretic response is less prompt and less forceful (76). A certain amount of the salt and water are retained; the body weight rises. Even in health the volume of the extracellular or interstitial fluids is quite elastic and varies with the amount of sodium salts and water available. However, water can not be retained without an equivalent amount of salt nor salt without an equivalent of water. The volume of the intracellular fluids appears to be far more constant, as it needs must be if function, which is presumably related to composition, is not to be continually disorganized. Because of the supposed impermeability of normal cell membranes to the basic ions, at least, of the intracellular fluids, it has been generally assumed that changes in the electrolyte concentration and osmotic pressure of these fluids are compensated by limited transfers of water to and from the cells. Such transfers have been demonstrated between blood plasma and cells in response to alterations of the reaction or electrolyte concentration of the plasma.

If, instead of sodium chloride, hydrochloric acid, or salts (ammonium chloride or calcium chloride) which contribute only the chloride ion, are administered, the chloride is rapidly excreted. The greater part of it is excreted in combination with ammonia, but a fraction carries with it sodium withdrawn from the body fluids,—with this sodium goes an equivalent amount of water (44, 123, 160). A comparable series of events follows the administration of acidifying sulfate salts (123). Acid ions are not retained except in combination with base. The administration of sodium bicarbonate or sodium salts of oxidizable organic acids (sodium lactate, acetate, etc.) causes the urine to become alkaline, ammonia excretion diminishes and the administered base is excreted, partly as bicarbonate, partly as sodium salts of the acids which would ordinarily have appeared in the urine in combination with ammonia. In some instances the sodium is not completely excreted until some time has elapsed and during this delay there may be fluid retention with a distinct increase of body weight. This would suggest that sodium bicarbonate had been retained. To a limited extent this may be the case. However, analyses have shown that part, at least, of the sodium retained, holds with it chloride (15, 40, 154). The retention of considerable quantities of bicarbonate alone would produce alkalosis and a dislocation of the normal electrolyte pattern. By the excretion of bicarbonate and other buffer acids and the retention of Cl, normally the predominant acid ion of the extracellular fluids, this disturbance of electrolyte and acid-base equilibrium is most effectively mitigated, sometimes at the expense of the interstitial fluid volume of the body. Reaction and detailed inorganic composition of the body fluids are not sacrificed entirely for osmotic pressure.

Nor are the volumes of the blood and extracellular fluids matters of complete indifference. Administration of large amounts of fluid of ideal composition,—i.e., as nearly as possible resembling a protein-free filtrate of plasma,—provokes a more equable eliminative response than does the administration of any less balanced fluid, and will, therefore, effect expansion of body fluids more regularly and for longer periods. Nevertheless, the extent and duration of this expansion are both limited. Attempts to reduce fluid volume by dehydrating measures are resisted by limitation of volume and increase of concentration of the urine.

In the preceding paragraphs the kidney may seem to have been pictured almost as the personal adjuster of the internal environment. The fact that the kidney is the ultimate channel for such adjustments justifies this treatment, however, without teleological implications of agency. To be sure, other channels such as the gut, the skin and the lungs may serve in the excretion of water and salts. These are not, however, their most important functions. In the distribution of water and salt in the body, besides diffusion and osmotic forces, local and general variations in the circulation must play an important part. In the discussion of the alterations of excretion and the changes of the volume of extracellular fluids brought about by the administration of salts and water to normal subjects these factors as well as the actual changes in the composition of blood and body fluids have been momentarily neglected in order to emphasize the salient excretory reactions occurring through the normal kidney. Qualitatively the same reactions are observed in subjects with famine edema or animals which have been subjected to plasmapheresis. In a quantitative sense the reactions are different. Schittenhelm and Schlecht (280, 281) found that large quantities of water swept salt out of patients with famine edema and sometimes caused profuse diuresis; salt without water was well excreted, withdrawing water from the body; salt and water given together exaggerated edema. The diuretic effects of either salt and water alone were often abnormally great, presumably because there was a large excess of salt and water in the body; the retention of salt and water given together was unusually large. All in all it has been possible to reproduce in famine victims and in dogs with depleted serum proteins all the abnormal responses to water, salt and alkali (154, 327) administration which have been reported in patients with nephritic edema and attributed to renal impermeability. However, the existence of anatomical changes comparable to those of nephritis has not been demonstrated either in severe malnutrition or in dogs which have been subjected to plasmapheresis.[4]

Volhard (320) and Elwyn (105, 106) have advanced against the Starling theory another objection. They claim that if edema were due simply to increased filtration there should be a demonstrable reduction of the plasma volume in nephrosis or other conditions associated with hypoproteinemia. A similar objection might be raised to any theory which ascribes edema to extrarenal disturbances, not excluding the theories of Volhard and Elwyn, themselves. The gist of their arguments appears to be that if there is increased filtration this must continue *ad infinitum*, whereas self limitation of a comparable tendency to redispose the fluids of the body in response to nervous, hormonal, secretory or other more vitalistic forces is more easily conceivable. The argument of Volhard and Elwyn against the Starling theory can have serious weight only if the interstitial spaces and serous cavities be considered as of infinite capacity, inelastic, and without other outlet than reabsorption through the vascular walls. The effect of reduction of the proteins on transudation and reabsorption is quantitative and measured by the degree of the protein deficiency. If this becomes unusually great new sources of resistance must be found in the elasticity of the tissues, perhaps in acceleration of the lymphatic flow, possibly in compensatory vasomotor reactions. There is no reason to believe that in this, like any other reaction, equilibrium will not be attained. The filtration theory only states that the chief determinants of this equilibrium point are the concentration of the serum proteins and the capillary blood pressure. As to the actual magnitude of the blood volume in nephritic edema it has already been pointed out that there is some question. Determinations by both dye and carbon monoxide methods agree that it is not unusually large (72, 73, 87, 89, 142, 190, 255), some observations by the carbon monoxide (325) method would indicate that it is usually slightly reduced. The Starling theory does not attribute control of blood volume to the serum proteins except in an extremely limited sense. Other factors remaining unchanged, reduction of the blood volume at the expense of the fluids only will, by increasing the protein concentration and the oncotic pressure of the serum, retard transudation of fluid to the tissues and accelerate reabsorption into the capillaries. Increase of blood fluids will have the opposite effect. However, such changes will be relatively transitory departures from an equilibrium point, if the subject has free access to exogenous fluids and salt, and the kidneys and vasomotor system, which appear to be the principal guardians of blood volume, react in the normal manner. Nevertheless, with extreme hypoproteinemia a condition may be reached in which the tendency to transudation is so great that acidifying diuretics or vomiting may lead to considerable hemoconcentration without effecting any large reduction of edema. This is well illustrated in cases reported by Peters, Wakeman, Eisenman and Lee (253). With serum proteins far below 4 per cent ammonium chloride produced little or no diuresis, although the blood water diminished, as evidenced by increased serum proteins and hemoglobin. With serum proteins above 4 per cent it was usually possible to evoke profuse diuresis by the same measures. These cases also afford an example of the fact that the edematous tendency due to serum protein deficiency can be wholly or partly combated by the influence of other forces that control the water and salt balance of the body.

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[4] Barker and Kirk (27) reported certain pathologic renal changes in dogs after prolonged plasmapheresis; but Leiter (184) and Shelburne and Egloff (294) did not confirm their findings. The subject has been discussed at some length by Leiter (184).

Although it does not seem to be necessary to refer the dropsical tendency as a whole to impairment of renal permeability for water, sodium chloride or any specific inorganic ion, consideration must be given to the alterations of water and electrolyte excretion and serum electrolyte patterns which accompany the edematous state. Much controversy has centered about the relative importance of water and sodium chloride in promoting edema. Since Strauss (304) and Widal (329, 330, 331) demonstrated the diuretic tendency of salt restriction it has been generally taught that water was retained only after sodium chloride, to dilute the latter. This view found support in the common experience that edematous patients often excreted without difficulty large amounts of water when given without sodium chloride (290, 320). In fact, not infrequently the water swept some previously retained salt from the body, giving rise to profuse diuresis. Interpreted strictly, these experiments only prove that water is not retained without an equivalent amount of salt. Similar clear cut experiments dealing with the effects on patients with nephritis of large amounts of salt without water are not available. The ingestion of water without salt, although not pleasant, does not usually provoke any serious distress. Large amounts of salt without water give rise to uncomfortable thirst, and may cause diarrhea to confuse interpretation of the experimental results. In famine edema Schittenhelm and Schlecht (279, 280) found that salt without equivalent amounts of water was well excreted and swept out with it a certain amount of water. The effects of both sodium chloride and water in a given case are somewhat dependent on the previous dietary regime of the patient, who does not differ qualitatively in this respect from the normal subject. Water is less likely to be retained if there is a deficiency of sodium chloride in the body; it is probable, also, that sodium chloride is less easily retained if water for its dilution is not available. Salt and water are inseparably connected and it would seem idle to speculate which is the primary agent. Analyses of the blood support this view. If either salt or water were retained in excess the total electrolyte concentration of the serum should depart from its normal level. Although individual cases may exhibit disturbances of total electrolyte concentration, such disturbances are not consistently observed in nephritic edema (see below).

Whether retention of water is the response to any given ion or salt has also caused much argument. It is generally agreed that potassium salts have no hydropigenous effects. Some, indeed, claim that they (41, 42, 146, 187) act as diuretic agents. This is true, not only of the conventional saline diuretics, but even of potassium chloride and bicarbonate. The different effects of potassium and sodium salts are generally ascribed to their distinctive distributions within the body. If potassium is the predominant base of cellular tissue, it could not be well retained unless edema affected the cells. It should, then, act as an indifferent substance in conditions affecting only interstitial fluids. This explanation is, of course, no more valid than the presumptions concerning the distribution of bases and the location of edema on which it is based. These, it has been pointed out, rest entirely on inferential evidence. That potassium has an actual diuretic effect is harder to explain and has been demonstrated with less certainty. If restriction of sodium chloride promotes diuresis, substitution for sodium chloride of an indifferent salt, an equivalent procedure, must have a similar effect; the diuresis, however, will be referable to limitation of sodium chloride, not to the indifferent salt. Many of the experiments in which, it is claimed, administration of potassium salts has led to diuresis, are of the substitution type and, therefore, not relevant to the subject. In experimental edema of dogs Weech (327) finds potassium chloride entirely indifferent, it is neither hydropigenous nor diuretic. Substitution of potassium chloride for sodium chloride has the same diuretic effect as simple withdrawal of sodium chloride. In other experiments potassium and sodium salts of different acids have been compared, making it difficult to distinguish the relative effects of the acid and basic ions (27a). Finally, the nature of the diuresis and the phenomena which accompany it are extremely variable. These matters will be discussed in detail below. At this point it need only be said that the evidence that the potassium ion has a specific diuretic effect is too suggestive to be denied, but not sufficiently unequivocal to be accepted.

A more serious point at issue is the relative importance of the sodium and chloride ions. The subject is doubly confused because of the unfortunate inadequacy of analytical data. The technical simplicity of chloride determinations led to their early and extensive application and the accumulation of much information concerning the behavior of the chloride ion in relation to edema. The difficulties attending sodium determinations have prevented comparable studies of sodium metabolism. Moreover, through much of the work on the subject there has been a tacit assumption that chloride was essentially a measure of sodium chloride and, therefore, indirectly, of sodium. On the basis of chloride studies alone v. Korányi (322), Straus (304) and Widal (329, 330, 331) came to the conclusion that chlorides were primarily retained, water being withheld secondarily to dilute them and thus prevent disturbances of osmotic equilibrium. It was, of course, assumed that sodium was also retained to neutralize the chloride. The discovery that sodium bicarbonate also caused water retention (40, 41, 164, 199), while the chlorides and sulfates of calcium and ammonium (24, 43, 123, 160) had diuretic effects and potassium chloride and bicarbonate were well excreted (41, 42, 43, 187), led Blum and others to conclude that it was the sodium ion only which promoted edema. This is not, however, an entirely obligatory conclusion. The chlorides and sulfates of calcium, magnesium, strontium and ammonium, by contributing only chloride or sulfates to the internal environment, produce a distinct acidosis. Administration of these salts is equivalent to the administration of hydrochloric or sulfuric acids. Retention of the acid ion would result in a severe acidosis, which has, in itself, a diuretic effect. It is, perhaps, significant that this diuretic effect is secured with greater difficulty and with the production of a higher degree of chloride acidosis in nephritic patients with extremely low serum proteins and a severe tendency to edema (245, 253). The sodium retention after bicarbonate administration is, as Widal first demonstrated, associated with retention of chloride (15, 41, 154); the bicarbonate ion is largely eliminated. The same is true, according to Ambard (15), of sodium sulfate: the sulfate ion is excreted, while some of the sodium is retained with a certain amount of chloride.

From these studies, the results of which differ only quantitatively from those described above as reactions of normal subjects and from the responses of subjects with malnutrition edema, it is possible to conclude that edema represents an extracellular retention of a solution which resembles in its composition a filtrate of plasma. Retention of water is facilitated by the presence of an available supply of electrolytes of the composition characteristic of such a filtrate. Such a salt mixture consists chiefly of sodium chloride; but contains sodium in excess of chloride ions. The failure to excrete the normal amount of urine or to unload through the kidneys any excess of fluid which may have accumulated in the tissues when the serum oncotic pressure is reduced, then becomes explicable without any assumption of renal impermeability. The plasma is normal, except for the protein deficiency, which tends merely to shunt the fluid from the blood stream to the interstitial tissues, leaving no stimulus to the kidneys for its removal. If, however, the oncotic pressure rises, the tendency to transudation diminishes, the fluid becomes available and diuresis results. Furthermore, if the composition of the fluid is altered the kidneys respond as they do in the normal subject, to restore osmotic pressure, acid-base equilibrium or any of the other vital constants of which they are natural guardians. If such reactions necessitate the excretion of water, diuresis follows. In this case the underlying cause of the edematous tendency is not affected; but a new point of balance is secured by disturbance of equilibrium from another direction.

It has been pointed out that a distinction must be drawn between the edemas of chronic nephrotic forms of nephritis and those which occur in acute nephritis or the terminal stages of chronic glomerulonephritis or arteriosclerosis of the kidneys. These last can usually be attributed to heart failure, although reduction of serum proteins may be a contributory factor. In the early stages of acute nephritis circulatory disturbances and increased permeability of the capillaries are chiefly responsible for the edematous tendency. There is also a possibility that in some cases of acute nephritis with extreme oliguria or anuria there may be true hydremic plethora and renal retention of salt or water or both (142, 211). How far this contributes to the edema is uncertain. In patients with obstructive anuria who have received the usual quantities of salt the chlorides of the serum have been found enormously increased without any signs of edema. Fishberg (116) and others report values exceeding 600 mgm. per cent of Cl. The lungs and skin appear to have excreted the water which was taken, but not the salt. What internal adjustments are made to compensate for such accumulations of electrolytes without equivalent amounts of water are still unknown. In extreme oliguria or anuria of acute nephritis the situation is more complex because of the presence of the vascular damage mentioned above, cardiac failure or serum protein deficiencies.

Such considerations seem to lead inevitably to certain conclusions: first, that reduction of serum proteins and cardiac or vascular disturbances afford quite adequate explanation for the occurrence of edema in nephritis; second, that water, salts and ions have effects essentially similar on fluid and salt exchange in normal individuals and in edematous nephritics; third, that the edema of nephritis is subject to the same influences as any other type of edema associated with equal serum protein deficiencies. To ascribe any importance in the production of edema to renal impermeability, or to alterations in the electrolyte patterns of blood and body fluids seems, at first sight, entirely superfluous. The problem is, however, actually not quite so simple. One may say that reduction of the oncotic pressure of the serum, changes in vascular permeability and heart failure are the chief recognized causes of nephritic edema and that the reactions to salts and water are qualitatively like those of other subjects with edema referable to similar extrarenal abnormalities. One can not deny that renal damage and disturbances of electrolyte patterns of body fluids may contribute to the edema of nephritis its characteristic stubbornness. The solution of the question ultimately requires among other things the collection of a greater body of well controlled experiments which will permit accurate quantitative as well as merely qualitative comparisons of the reactions of subjects with various types of edema.

NEPHRITIC ACIDOSIS

v. Jaksch (321), in 1888, found that in the terminal stages of nephritis the titratable alkalinity of the serum was greatly reduced. He concluded that uremic coma was, like that of diabetes, a result of acid intoxication. Since that time it has been demonstrated by numerous observers that both the hydrogen-ion concentration and the bicarbonate content of the blood might be greatly reduced in nephritis (23, 25, 77, 81, 99, 140, 150, 206, 236, 242, 251, 267, 272). This acidosis is usually observed only when renal function is extremely impaired and when there is obvious nitrogen retention (25, 81, 140, 150, 206, 251, 272). It is, however, not uncommonly encountered in acute nephritis (25,140) and, on the other hand, may fail to appear until the very point of death. It is seldom seen in the nephrotic types or stages of the disease (25, 251, 272). Many besides von Jaksch have held it responsible for the uremic syndrome. There is little reason, however, to believe that it is the direct cause of any of the symptoms of this state except dyspnea.

Its causes will be discussed in detail later. Chiefly they appear to be: (*a*) diminution of the ability of the kidneys to excrete ammonia, so that the organism is forced to utilize its fixed bases for the neutralization of urinary acids; (*b*) impairment of the capacity to excrete a highly acid urine, limiting the value of buffer acids in the preservation of the body's base supplies; (*c*) the accumulation in the body of excessive amounts of phosphoric, sulfuric and probably organic acids.

THE TOTAL SALT CONTENT OF THE SERUM (FREEZING POINT AND CONDUCTIVITY)

The first means employed for the estimation of the concentration of total solutes in the serum was determination of the specific gravity. This is, however, so variously affected by different solutes and so dependent upon the protein content, that it is unadapted to this purpose. That specific gravity is influenced chiefly by the concentration of protein was recognized by Bright. Recently Moore and Van Slyke (223) have shown that the relationship is so close that the concentration of protein in the serum can be estimated with reasonable accuracy from the specific gravity, at 20°C., by the equation,

$$\text{Gram per cent of protein} = 343\ (\text{specific gravity} - 1.007)$$

v. Korányi (322, 169) and his associates introduced determination of the freezing point. (The difference between the freezing point of an aqueous solution and that of distilled water, Δ, is a measure of the concentration of dissolved molecules and ions in that solution and, therefore, of its osmotic pressure.) The freezing point depression of normal human serum, Δ, is according to v. Korányi (322) and other observers $-0.56° \pm 0.02$. In patients with ablation or severe impairment of renal function, v. Korányi found Δ greatly increased, sometimes to more than $-0.70°$. These increases he attributed to retention of waste products by the kidney, although he was unable to correlate them with the level of non-protein nitrogen or of other solutes which he determined.

With a somewhat different aim, others have measured the specific conductivity of serum (25, 101, 130, 131, 287). This is supposed to give an estimate, not of the total concentration of solutes, but of the concentration of ionized solutes, or electrolytes. In contradistinction to the freezing point depression, which is so often increased in nephritis, the conductivity is more commonly lowered (25, 101, 130, 131). Moreover, it is especially in those cases with the greatest freezing point depression, cases with advanced renal insufficiency and retention of nitrogenous waste products, that conductivity is most frequently and greatly reduced (130). This probably explains the failure of v. Korányi to demonstrate a direct relation between freezing point depression and nitrogen retention. Deficiency of electrolytes is commonest in those sera which contain an excess of non-electrolytic solutes. This inverse relation has led some to conclude that reduction of serum electrolytes is a protective response of the organism to maintain the osmotic pressure of the body fluids constant in the face of accumulation of organic compounds. In this case the organic compounds are nitrogenous products. The same argument has, however, been advanced to explain the similar electrolyte deficiencies of diabetes, in which case the offending organic compound is glucose. In neither case has it been possible to demonstrate any exact correlation between the non electrolyte increases and the electrolyte deficiencies (251) nor has it been possible to demonstrate a compensatory response of this nature experimentally. Hartmann (140), reversing the usual position, has suggested that non-protein nitrogen retention in nephritis is a reaction to compensate osmotically the electrolyte deficiency. This attitude appears even more untenable than the other because it denies the agency of the kidney in the production of the only blood abnormality for which it seems unequivocally responsible.

The chief retention product in nephritis, urea, can traverse all cell membranes and distributes itself in even concentration throughout the fluids of the body. Accumulations of such a substance do not disturb osmotic equilibrium between any two media in the organism. On the other hand cell membranes have selective permeability for inorganic ions and are especially impermeable to bases. For this reason, if the concentration of base in any one of the media is altered, osmotic equilibrium can be established only by exchange of water between this and adjoining media, an exchange which entails changes of volume. The process is well illustrated by the action of red blood cells in salt solutions of various concentrations. From the standpoint of osmotic relations within the body it is hard to see how freely diffusible non-electrolytes and electrolytes with conditioned diffusibility can play reciprocal compensatory rôles.

TOTAL ELECTROLYTES OF THE SERUM

In nephritis the concentration of the various inorganic components in serum and other extracellular fluids may be profoundly altered and these alterations must be taken into consideration in any discussion of the electrolyte metabolism. The variety, frequency and inconsistency of the electrolyte disturbances can be seen from the frequency charts in figure 1 which is constructed from data collected by the author and his associates during the last eight years. The results are generally similar to those which have been reported by Salvesen (272), Atchley and Benedict (23), Sunderman (308) and others. They include data previously published by the author (251).

TABLE 1

	BASE		Cl		HCO_3		INORGANIC P	
	Observations	Cases	Observations	Cases	Observations	Cases	Observations	Cases
Destructive kidney conditions:								
a. Chronic glomerular nephritis (vascular interstitial type)	106	26	166	41	164	40	129	29
b. Arteriosclerotic renal disease	37	17	59	24	60	24	42	19
c. Other bilateral destructive diseases of the kidneys	42	13	49	15	44	16	50	13
Total	185	56	274	80	268	80	221	61
Acute nephritis	50	22	72	35	61	29	56	27
Nephrotic syndrome	111	16	132	19	126	18	129	19
Grand total	346	94	478	134	455	127	356	107

In this chart the abscissae represent the concentrations of each individual component in terms of univalent combining equivalents. This mode of expression has been chosen because it permits comparison of the relative effects of the alterations of each constituent on total

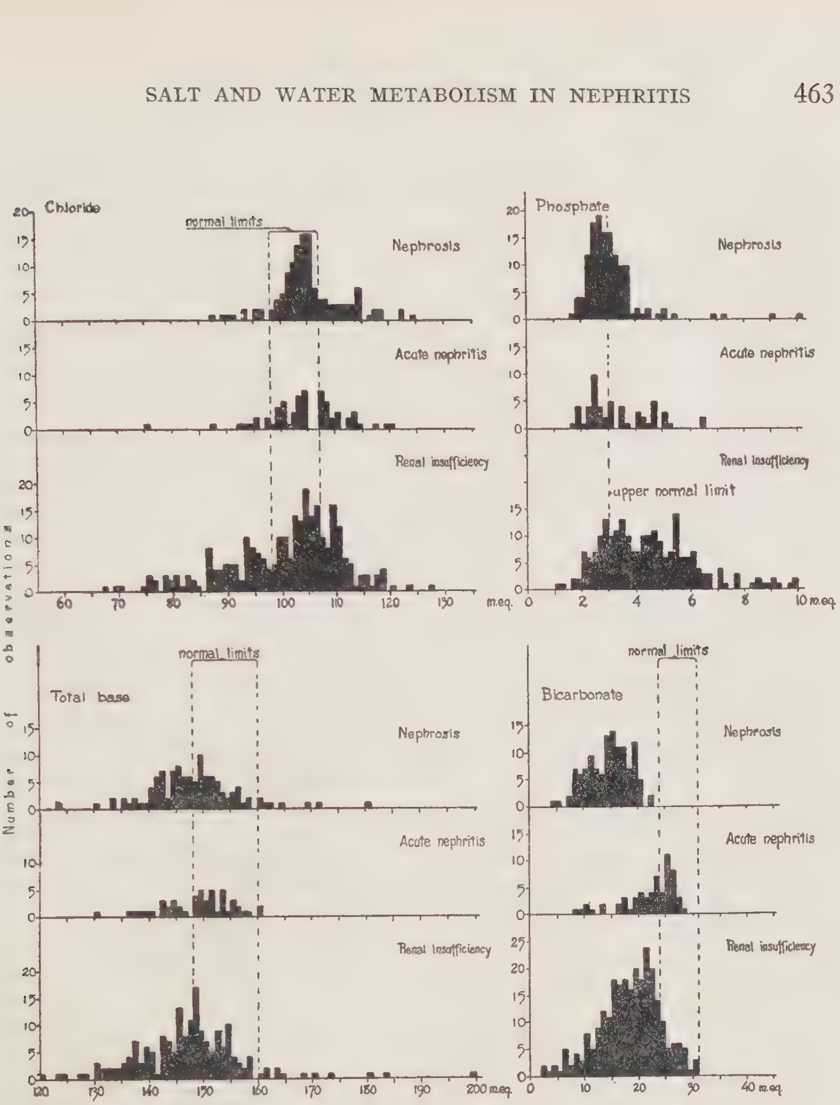

Fig. 1. The Concentration of Total Base, Chloride, Bicarbonate and Inorganic Phosphate in the Serum of Patients with Renal Disease

The abscissae in each case represent base combining power in milliequivalents, ordinates the number of observations. The limits of normal variation are indicated by broken lines. Cases have been divided into three groups, which have been defined in the text: (1) Those with the nephrotic syndrome; (2) those with acute nephritis; (3) those with advanced renal insufficiency due to chronic glomerular nephritis, arteriosclerosis or other bilateral destructive kidney diseases.

acid-base and electrolyte equilibria.[5] The cases have been divided into 3 groups. The character of the material can be better appreciated from the table 1. In the first group, "destructive kidney conditions," are included cases in which there were distinct evidences of chronic renal insufficiency, for the most part patients in the terminal stages of kidney disease. In the charts subdivision into the three component groups has not been attempted, because the electrolyte disturbances in all the groups are of the same general nature, varying only in degree with the duration and severity of the disease and its attendant symptoms and complications (251). This may be considered as evidence that they are more or less closely connected with severe renal insufficiency.

In the third group, "nephrotic syndrome," are included cases of glomerular nephritis, amyloid disease, and others of unknown origin. All presented profuse albuminuria and tendency to edema, with little or no renal insufficiency or signs of vascular disease. Etiological distinctions can be connected with no characteristic disturbances of electrolyte patterns (253). Some of the cases in the first group, during certain periods in their course, exhibited clinical pictures which were predominantly of the nephrotic type. Observations during these periods should, perhaps, be included in the "nephrotic syndrome" group. Such instances, however, are not frequent.

The most striking feature of the chart is the variety and inconsistency of the findings in each clinical group and in every electrolytic component.

TOTAL BASE

In a solution as nearly neutral as serum there can be no appreciable excess of either free acid or free base; all the acids and bases must be present in the form of neutral salts. Blood contains only insignificant quantities of ammonia and organic bases; all its acids are neutralized by the inorganic elements, Na, K, Ca and Mg. Determination of the sum of the combining equivalents of these elements affords a measure not only of the total base, but also of the total electrolyte concentration of serum. The provision of methods for the determination of total base by a single procedure has given rise to an increasing volume of work on the total electrolyte concentration of serum in disease. These studies confirm the impression gained from earlier determinations of conductivity; total base and electrolytes are far more often reduced than increased (23, 61, 77, 140, 206, 230, 251, 272). These reductions are most frequent and severe in the terminal stages of chronic nephritis, especially in patients with alkali deficits ("acidosis")[6] (23, 140, 251, 217, 272). In such cases serum total base may be 15 or 20 per cent below normal; values below 130 milliequivalents (normal = 150 to 156) have been reported. It is this base deficit which appears to be chiefly responsible for the alkali deficit of terminal nephritis (23, 77, 206, 251). The causes of the base reduction seem to be: (*a*) impairment of the ability of the kidney to form ammonia, necessitating the excretion of more than the usual quantities of fixed bases to neutralize acids in the urine (200, 236, 258, 259, 272, 315); (*b*) impairment of the power to excrete a highly acid urine, which limits the base-sparing action of the kidney in another dimension (315); (*c*) anorexia and vomiting, which prevent repletion even if they do not directly deplete the base stores of the body (251); (*d*) loss of the tendency of the kidney to limit the excretion of salt in the presence of serum salt deficiency (230, 252). Salvesen (272) and Peters, Wakeman, Eisenman and Lee (252) and others (217) have found that the vomitus of patients with terminal nephritis contains little or no free hydrochloric acid. Therefore persistent vomiting causes both base and alkali deficits in nephritis, which differs in this respect from other vomiting states in which loss of free hydrochloric acid results in alkali excess. In the hyposthenuria of the end stages of renal disease the kidney fails to restrain the excretion of chloride in the urine when serum chloride falls. Relatively large amounts of chlorides have been found in the urines of nephritic subjects when the serum Cl was far below the level which Ambard (15) considered as the normal chloride threshold and below the level at which, in pyloric obstruction, the urine usually becomes chloride free (230, 252). Although a similar failure to restrain the excretion of base has not been demonstrated by direct analyses, it is safe to say that with impairment of ammonia formation this chloride must carry with it an equivalent amount of fixed base.

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[5] The factors by which the analytical data are converted into terms of base combining equivalents have been described in detail by Peters, Wakeman, Eisenman and Lee (251). For the calculation of bicarbonate from the observed CO_2 content of serum, it has been assumed that the serum pH is, in every case, 7.35. Milliequivalents of HCO_3 can be converted to volumes per cent of CO_2 by the formula 2.37 m.eq. = volume per cent. It is also assumed that four-fifths of the inorganic phosphate of serum is in the dibasic form, B_2HPO_4, one-fifth in the monobasic form, $BHPO_4$. These are the proportions in which the two forms exist at pH 7.35. Milliequivalents of inorganic P, can, therefore, be converted to milligrams per 100 cc. by the formula $\frac{31.03}{18}$ m.eq. = 1.72 m.eq. = milligram per 100 cc. For Cl 3.55 m.eq. = milligram per 100 cc.

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[6] The terminology here employed for the definition of disturbances of acid-base equilibrium is that proposed by Peters and Van Slyke (250).

(1) *Base deficit* and *base excess* indicate conditions in which the total base of the serum is lower or higher than normal.

(2) *Alkali deficit* and *alkali excess* indicate that serum bicarbonate is lower or higher than normal because of some disturbance which has affected primarily the concentration of bases or non volatile acids,—e.g., the alkali deficit of diabetes or the alkali excess of pyloric obstruction.

(3) *CO_2 deficit* or *CO_2 excess* indicate that serum bicarbonate is lower or higher than normal because of some disturbance which has affected primarily the concentration of free carbonic acid in the blood,—e.g., the CO_2 deficit of overventilation or the CO_2 excess of emphysema.

The relative frequency of base reductions in patients with severe renal insufficiency is well illustrated in figure 1. Out of 185 observations, total base concentration was below the low normal limit of 148 milliequivalents 97 times and less than 140 milliequivalents 39 times. Although such base deficiencies are more common in the most severe cases and, when extreme, are of grave clinical significance, they can not be correlated with any of the more direct evidences of renal insufficiency such as the blood non-protein nitrogen. The reasons for this failure of correlation are obvious when the conditions under which base deficits develop are analyzed. In a large proportion of instances persistent vomiting is an important symptom and plays a large rôle in base depletion. The lowest values are usually observed in vomiting patients. When there is no active vomiting, nausea may be so distressing that no salt is taken. The result of such voluntary abstention from salt is quite similar to that of therapeutic restriction of sodium chloride. The latter almost inevitably leads to base depletion, which is accelerated by the production of water diuresis (252). The ability of the organism to preserve its salt stores, by restraining excretion through the kidneys is lost. Usually there is a concomitant loss of body weight. The tendency to preserve osmotic pressure by sacrificing water with base is not entirely lost. However, even if edema develops as a result of heart failure, base may remain low (251). In extreme cases even the most vigorous parenteral administration of salt will not prevent base depletion (252). Concerning the channels through which the base escapes there is little direct evidence. From studies of chloride balances one can infer that the chief channel is the kidneys. The quantities in vomitus may be of considerable importance when emesis is severe. Excretion by the bowel is also increased in some instances for unknown reasons (61, 251).

In rare instances base excess is encountered (67, 230, 251). The causes of this unusual disturbance are not entirely clear. In the cases of Peters, Wakeman, Eisenman and Lee (251) the base excess was a transitory state which occurred when patients had received comparatively unregulated diets. Altogether high base was observed in only 11 out of the 285 observations in figure 1. In 8 of these the observations were made in the dispensary or immediately after the patients had entered the hospital. Another appeared during an intercurrent erysipelas. In 4 of the patients subsequent examinations revealed normal or low figures. In one case base fell rapidly during water diuresis without salt restriction. This suggests that large quantities of base can not be excreted unless large amounts of water are also made available.

Although calcium suffers somewhat, it is sodium which bears the chief brunt of the base reduction; potassium is more often above than below normal. It is, furthermore, the fractions of sodium which are combined with chloride and bicarbonate which are chiefly diminished.

In nephrotic states with edema less important base deficits are observed with some regularity (10, 61, 140, 207, 253, 272). For these other causes must be found than those to which the base deficits of advanced nephritis have been ascribed. Ammonia production and acid excretion appear to be unimpaired in the degenerative stages and forms of nephritis (200, 272); vomiting is not a characteristic feature; the kidneys show no unusual tendency to permit escape of salt from the body. The base deficit may be associated with the serum protein deficiency; that is, it may involve only that fraction of the base which is usually combined with the proteins. It is, however, impossible to demonstrate an exact correlation between serum proteins and base concentrations in the author's data or those of other observers. Base, to be sure, tends to be low when proteins are low; but this is a mere restatement of the fact that base deficit is common in nephrotic states with edema. In dogs after plasmapheresis Darrow (88) has sometimes found slight reductions of base; but the correlation between proteins and base leaves much to be desired. Studies of the level of serum base in famine edema and malnutrition might throw further light on the subject. Such data as have been collected by the author are confused by the effects of complicating symptoms connected with the clinical conditions from which the patients were suffering: diabetes, vomiting, diarrhea, severe infections, etc. All of these conditions can give rise to base deficits even before they have persisted long enough to cause wasting and hypoproteinemia. In pneumonia a condition is found in which base is generally low in the absence of either demonstrable renal insufficiency or hypoproteinemia (307). At present one can go no further than to admit the fact that there is in pneumonia and other severe febrile infections a disorganization of the usual electrolyte balance of the organism which evidences itself in a tendency for more than the usual amount of water to be retained in the interstitial fluids in proportion to electrolytes. The facts in nephrosis can be stated in the same terms without implications concerning the cause of the abnormal state.

For the most part the reductions are slight. Values below 140 milliequivalents are rare. Of the 11 indicated in figure 1, the lowest, 123 milliequivalents, was seen in a patient in the last stages of tuberculosis with amyloid disease and was probably in part referable to diarrhea. Another 3 were observed in a nephrotic subject with predominantly gastrointestinal symptoms, persistent vomiting and diarrhea; 1 in the last days of life of an extraordinary case complicated by arthritis, heart disease and profound anemia, who also suffered from persistent vomiting and diarrhea. The remaining 6 were all from 1 patient with nephrotic glomerular nephritis, who has been studied over a period of more than four years. Although vomiting has been, at times, a distressing symptom in her case also, the lowest base values do not coincide with the periods of most profuse vomiting. Moreover, chlorides have remained normal even when base was greatly reduced. It seems doubtful, therefore, whether the base deficits can be ascribed entirely to emesis.

Base excess is rarely encountered in patients with the nephrotic syndrome (253). In figure 1 it appears only 5 times in 2 patients. These two are distinguished from the others of the series by the fact that on no occasions during the periods of observation did they present serum base deficits. Nevertheless, it is not possible to connect this distinction with any peculiar clinical characteristic. One of the patients had a glomerular nephritis with a considerable serum protein deficiency, continuous microscopic hematuria and a rather stubborn tendency to edema; the other had in all probability an amyloid nephrosis with no hematuria, slight serum protein deficiency and edema that disappeared rapidly under dietetic treatment.

Whether during the edematous stages base is low or high, it tends to approach the normal concentration with clinical improvement and disappearance of edema, even while albuminuria and serum protein deficiencies persist.

In acute nephritis serum base deficit is not uncommon during the active stage of the disease. It is not unnatural that it should attain a higher frequency and degree in the most severe cases and therefore show a rough parallelism to nitrogen retention. One is not entitled on this account to draw any inferences concerning its origin. As acute nephritis may exhibit all the phenomena of nephritis, varying from an almost purely nephrotic syndrome to the picture of terminal nephritis, it seems reasonable to ascribe the base disturbances to factors similar to those which are operative in the various chronic forms of the disease. It is quite possible that the serum electrolyte pattern is modified in some cases by the presence of an active systemic infectious disease. It is also probable that, in extreme oliguria or anuria, there is actual retention by the kidneys of water and salt.

The effects of salt administration on base levels can be more satisfactorily considered in the discussion of the individual bases.

Concerning the concentrations of total base in tissue cells in nephritis one can only conjecture. Studies of the distribution between red blood cells and serum have revealed the same relations in nephritic as in normal blood (103, 145, 229). The same is true of the distribution between serum and transudates (126, 133, 144, 194, 244). Osmotic equilibrium has been proved to persist between media of the internal environment which are accessible to analysis. It seems a not unreasonable inference that it is maintained also in all other media.

If, as is generally believed, the membranes of tissue cells, like those of red blood cells, are impervious to bases, osmotic and electrolyte equilibrium could be achieved, in the face of a change in the base concentration of serum and intercellular fluids, only by transfer of water to or from the cells. One would expect, then, to find the cells swollen when serum base was low, shrunken when serum base was high. It is obviously impossible by direct experiment to ascertain the relative size of tissue cells. Eisenman (103) has made a large number of simultaneous determinations of hemoglobin (oxygen capacity), blood cell volume and total serum electrolyte balance in patients with a great variety of diseases. In this study the ratio, oxygen capacity: cell volume, serves as a measure of the relative water content of the cells. No consistent correlation could be found between this ratio and serum base concentration in nephritis or other diseases, although oxygen capacity: cell volume ratios were extremely variable in patients with abnormal serum base concentrations. On the other hand, Eisenman (103), like others (145, 229), has found the distribution of base between the water of cells and of serum quite constant, regardless of the serum base level. This can only mean that when serum base is altered osmotic compensation between blood cells and serum is secured by loss or gain of base, with or without water, by the cells. Whether or not, in this respect, tissue cells react like blood cells remains a matter of doubt. Alterations of potassium excretion which so often accompany changes of the sodium content of the body may be evidences of such compensatory reactions on the part of the cells.

SODIUM

In general, what has been said of total base holds equally well for sodium, because the latter makes up more than 90 per cent of the base of serum and extracellular fluids. Serum sodium is frequently low in terminal stages of nephritis with advanced renal insufficiency (25, 31, 171, 206, 232, 260, 272, 273), in the nephrotic syndrome (175, 176, 207, 272, 273) and in acute nephritis; abnormally high values are occasionally seen (66). The general causes of these disturbances have been discussed in the preceding section. What is of more specific concern in relation to sodium is the distribution of this element within the body and its connection with edema.

It has, from time to time, been claimed that in nephritis sodium salts may accumulate in certain tissues in extremely high concentration without proportional amounts of water. The evidence on which these claims are based is of two kinds: experiments in which large positive or negative salt balances have occurred without corresponding changes of weight or water balance; analyses of tissues. Most of the balance experiments must be discounted because of the failure to examine excreta other than the urine; others because they do not include analyses of serum. That the urine is not the only channel for the excretion of sodium was clearly demonstrated by Carl Schmidt's (285) studies on epidemic cholera, dysentery, and profuse catharsis. Fluctuations in fecal excretion of salt may occur even without diarrhea. Boyd, Courtney and MacLachlan (61) found increased fecal excretion of base in nephritic patients, especially those with edema, who were excreting less than the normal quantities of base in the urine. Peters, Wakeman, Eisenman and Lee (251) recovered large quantities of chloride, presumably combined with sodium, from the feces of a nephritic subject, without diarrhea, who received nothing by mouth, but was treated by subcutaneous and intravenous injections of saline. This case is especially important because it proves that the excessive excretion of salt from the bowel is not merely an expression of faulty absorption. Losses in vomitus may be unusually large because of the achlorhydria mentioned above (252, 272). Appreciable quantities may be eliminated in the sweat. Fluctuations in the sodium concentration of serum and extracellular fluids may account for negative or positive balances of variable magnitude. This is especially true during the development of, or the recovery from, base depletion. Efforts to evaluate the possible magnitude of the effect of such alterations of sodium concentration on sodium balances are frustrated by our complete ignorance of the actual volume of the intercellular fluids in normal persons, and are only worse confused in the presence of edema.

If sodium is segregated in high concentration in special repositories in nephritis, these repositories are not the transudates or the normal fluids accessible to analysis. Comparison of such fluids with serum has invariably revealed complete electrolyte and osmotic equilibrium (126, 133, 144, 194, 244). One must assume, then, that the sodium accumulates in certain tissue cells. Blum and Grabar (50) have presented analyses showing extraordinary increases of sodium in the brain, kidney, liver and heart muscle of patients who had died of nephritis. If these resports are accepted at their face value one is forced to conclude that there is an extreme disorganization of cell composition that permits the displacement of cellular potassium by sodium. To conclude that the cells accumulate the sodium in addition to their usual load of potassium would imply that osmotic equilibrium between the cells and the surrounding fluid is destroyed, which is tantamount to saying that the cells have lost their permeability to water. The materials analyzed, of course, consisted of intercellular fluid as well as cells. The high sodium content might be due to the presence of an unusually large quantity of extracellular fluid. The normal values for water content of the tissues and the great magnitude of the sodium increments invalidate this explanation. Blum, Delaville and van Caulaert (44) believe that in edematous states, at least, a certain part of the sodium and chloride in the body exists in a colloidal state. In some states of diabetic acidosis, hydropigenous nephritis and anaphylactic shock they found the ultrafiltrability of the sodium and chloride of the serum altered (45). This is at variance with other published studies (133, 144) and can not be accepted as satisfactory proof of the colloidal nature of sodium and chloride in either body fluids or tissues.

Davison and Salinger (90) found sodium high and chloride low in the blood cells of children with nephrosis. This observation has not been verified by others (103).

With the multiplication of published data dealing with base metabolism there has been an increasing tendency of late to direct the responsibility for edema from chloride and water and to place it upon sodium. Some aspects of the subject have been mentioned in the discussion of the causes of edema above. In general those who would incriminate sodium particularly, whether or not they introduce concepts of renal permeability into their arguments, rely upon the same evidence for support. Potassium salts do not cause edema and may even provoke diuresis, while sodium salts of the same acids induce water retention (41, 42, 43, 187). The chlorides of ammonium, calcium and magnesium have a diuretic effect (24, 45, 123, 160). The bicarbonate as well as the chloride of sodium exerts a hydropigenous action (40, 41, 164, 199). Diuresis is attended by excretion of sodium (43, 123); during edema formation sodium is retained (42). Blum and van Caulaert (43) have taken an extreme position, claiming that the chloride ion is dehydrating, while the sodium ion is hydropigenous. They state that the edema produced by sodium chloride is associated with serum sodium excess and chloride deficiency, in direct contradiction of the observations of the majority of clinical investigators cited above.

It has already been pointed out that, as edema fluid is a colloid-free filtrate of serum, it will assume the composition of serum. Potassium could not be retained without altering the whole nature of the interstitial fluids unless the accumulation of edema were intracellular. The chlorides of ammonium, calcium and magnesium contribute chiefly the Cl ion to the internal environment and this can not be retained without a proportional amount of sodium without the production of a severe alkali deficit. Sodium bicarbonate can not be retained as such without producing alkali excess. It has been repeatedly demonstrated that when water retention is provoked by bicarbonate the chloride balance becomes positive (15, 40, 154). In other words bicarbonate is not retained entirely as bicarbonate. Either the sodium of the bicarbonate finds for itself chloride from other sources or else the bicarbonate is excreted while a certain amount of sodium chloride is retained. Moreover, sodium bicarbonate does not invariably aggravate edema and may even have a diuretic action in individual cases (10). Potassium chloride, on the other hand, may have no diuretic effect and may induce water retention (184). It does not follow that potassium chloride is withheld. In one edematous case studied by Leiter (184) single doses of 4 grams of potassium chloride on two occasions were followed by diminished excretion of sodium. In both instances the proportion of added Cl recovered in the urine fell short of the potassium. The patient seems to have retained sodium chloride.

The acidifying chlorides may produce little or no diuresis. In this case the chloride ion heaps up in the blood at the expense of bicarbonate (253). However, it is combined in the body with sodium, not ammonium, and the salt which is actually retained is sodium chloride. Reported results of the effect of acidifying chloride diuretics in nephritis vary greatly. Gamble, Blackfan and Hamilton (123), in one case with edema, were able to produce profuse diuresis by means of ammonium chloride, calcium chloride and ammonium sulfate on separate occasions. In the intervals the edema reaccumulated. On every occasion during diuresis large amounts of base, chiefly sodium, were excreted, combined for the most part with the foreign acid. In a patient without edema little sodium was swept out. Linder (189) found that hydrochloric acid, when given to a nephrosis patient without edema, withdrew little sodium from the body, but was excreted chiefly as ammonium chloride. Gamble, Blackfan and Hamilton (123) concluded that acidifying salts caused sodium diuresis when there was a large amount of this ion available for excretion. In their edema case evidently the large stores of sodium in the transudates were available. In others with edema the sodium is not mobilized into the urine by acidifying measures. In one of the author's cases (253) with the nephrotic syndrome, mentioned above, ammonium chloride had no diuretic effect on two occasions when the serum proteins were below 4 per cent, but caused profuse diuresis when they had risen above this level. There were no striking differences in the serum electrolyte reactions on the three occasions. Apparently sodium can be unavailable for excretion for two reasons: either because with the proteins low it can not be mobilized from edema transudates; or because the general stores in the body have been depleted by salt deprivation. Linder's (189) case had been maintained for a period preceding the administration of hydrochloric acid on a salt poor régime and was free from edema. The excretion of ammonium rather than sodium in this case may be considered only as an attempt to protect depleted sodium stores.

Careful consideration of the evidence, then, leads to the conclusion, already suggested in the section on edema, that edema fluid is made of the materials of which interstitial fluids are composed and edema is aggravated by the provision of such materials in the proportions in which they appear in the interstitial fluids. Within certain limits the electrolyte pattern of the serum can be disturbed without provoking a diuretic response. When, because the proteins are extremely low, the forces which withdraw fluid from the interstitial spaces and render it available for excretion by the kidneys are weakened, an unusually great disturbance appears to be required to induce diuresis. That sodium should be retained in excess of chloride (46) follows from the fact that it exists in greater concentration in the serum and extracellular fluids, which contain more than enough sodium to neutralize both bicarbonate and chloride. Whenever a large retention of sodium is provoked, however, chloride is also retained, because the major fraction of sodium in the body is combined with chloride.

What determines the abnormal electrolyte patterns of the serum of patients with nephritic edema is unknown. The abnormalities may persist over long periods in spite of changes of treatment, although the latter may cause temporary disturbances. High chloride and low sodium pictures encountered in edematous stages when serum proteins are extremely low may remain after the proteins have risen far enough to permit diuresis (244, 253). Laudat and Grandsire (181) found that administration of a salt-poor diet in two nephritics resulted in reduction of the chloride without change of the sodium of serum. In edematous cases such a reaction does not invariably follow salt restriction (244, 253); such treatment may leave the serum electrolyte picture comparatively unaltered. One may ascribe the altered electrolyte pictures to the renal injury. The presumption then is that the regulatory mechanism of the kidney becomes disorganized by the disease in such a manner that it tends to maintain the concentrations of the various blood components at unusual levels. This concept would hold the kidneys responsible for the separate retention of individual electrolytes. In this case one is forced to the inevitable conclusion that base, which is usually low in the serum, is excreted more easily than water and chloride, which are usually high. It seems more intelligent to accept the simple fact that nephritic edema is associated with an abnormal serum electrolyte picture, without any unwarranted deductions concerning the mechanism by which this is produced and maintained and without ascribing to it an important part in edema formation, which appears to proceed independently of the electrolyte pattern in accordance with the serum protein concentration.

One can not, of course, deny that the abnormal electrolyte patterns may be evidences of a disorganization of the forces that regulate the constitution of the internal environment, among which renal activity holds a prominent position. In this sense, as has already been suggested above, they may be contributory causes of the edema, responsible for its peculiarly stubborn character. The causes of the electrolyte disturbances and their connection with the renal pathology remain mysterious. There is something peculiarly unphysiological, from the point of view of any one of the various theories of kidney function, in the term "renal impermeability," satisfying as it may sound to the unthinking ear, as an explanation of the failure of the organ to excrete individual solutes. The term could apply only to conditions in which a large proportion of the excretory units were destroyed or obstructed, a situation quite at variance with general conceptions of the pathologic picture in the nephrotic syndrome and one which would result in the retention not of one, but of all urinary solutes.

POTASSIUM

Information concerning the state of potassium in the body in health and disease is extremely limited and based almost entirely upon presumptive evidence, because of the fact that the greater part of the potassium is stored in the inaccessible intracellular spaces. Serum and extracellular fluids contain only small quantities which may represent a continuous load in process of transportation to and from cells, a sort of transportation reservoir. The concentration of potassium in normal serum is usually given as 4 to 5 milliequivalents (16 to 20 mgm. per 100 cc.). Some recent analyses (23, 94, 307) would suggest that these values are somewhat too high, possibly because delay in separation of cells from serum permitted potassium to escape from the former. In transudates somewhat smaller amounts have been found, the difference being apparently related to the difference in protein concentration. Diffusion experiments have, however, demonstrated that serum and transudates are in osmotic equilibrium in respect to potassium.

In nephritis serum potassium is usually normal (25, 59, 96, 168), although in the terminal stages of the disease it may be moderately elevated (66, 231, 232, 233, 260, 272). These accumulations have been ascribed, like all others, to a selective impermeability of the kidneys and have even been held responsible for the uremic syndrome by Stadthagen (300) and Voit (319). That potassium poisoning is the cause of uremia is highly improbable, both because the symptoms usually associated with uremia do not resemble those of potassium poisoning and because potassium accumulations do not occur with any consistency in uremic cases. That the accumulations in the serum are the direct result of renal impermeability has not been established. Boyd and Courtney (59) found negative potassium balances in 13 children with nephritis who were receiving diets that caused positive balances in normal children. All of these had normal serum potassium. Comparable studies on patients with high serum potassium have not been reported. Such studies as have been made have shown that nephritic patients excrete administered potassium well, although, when it is given in the form of the chloride, the chloride ion may be partly or wholly retained (184, 296).

It has already been mentioned in the section on total base that when serum base is deficient the base of the blood cells is comparably reduced without consistent expansion of the water content of the cells. Furthermore, the ratio of base concentration in cells to that in serum, in relation to water, is preserved in all pathological conditions which have been studied, including nephritis. Unless it shall be proved that there is in these conditions a disturbance of the normal distribution of potassium and sodium between cells and serum, the cells must, when there is a serum sodium deficiency, give up some of their potassium to preserve their normal size and contour. If the blood cells may be considered typical of tissue cells, the latter should react in a similar manner. Salkowski (270) found that potassium excretion increased in all conditions which caused destruction of tissue. Gamble, Ross and Tisdall (125) in experiments on the effects of starvation and dehydrating measures concluded that the latter did not deplete the potassium stores of the body unless they also caused tissue destruction. They concluded that intracellular water and salts were more solicitously protected than the extracellular stores of the same components, which served as a more or less elastic reservoir, a fluid and salt buffer against intracellular disturbances. In their starvation experiments they recognized that the loss of potassium was relatively somewhat in excess of the nitrogen excretion. This they assigned to water which had been held by glycogen. Similar losses of potassium have been reported more recently in experiments in which there has been no reason to suspect glycogen destruction. Moreover, the long accepted theory that glycogen retains water has been seriously questioned (65). Therefore it appears possible that the protection of intracellular water and potassium may not be quite as complete as Gamble's (125) fasting experiments suggested. Certainly in nephritis diuretic measures that drive out sodium do not leave potassium entirely untouched and the amounts of potassium excreted are too large to have emanated entirely from the interstitial fluids (125, 189). The latter, nevertheless, do seem to bear the brunt of all attacks on the body water stores and it is sodium among bases which is chiefly affected by measures which primarily influence the water exchange.

MAGNESIUM

According to available studies serum magnesium remains unaltered in nephritis (59, 260, 272). The metabolism of this element in disease has received little attention. Boyd and Courtney (59) detected a tendency to negative magnesium balances in nephritic children.

Magnesium sulfate has long been employed for its cathartic effect in nephritis, to promote the elimination of water and solutes by the bowel. Gamble, Blackfan and Hamilton (123) have shown that it acts as an acidifying diuretic; although, for these purposes it is less useful than salts which do not produce diarrhea. For the treatment of convulsive seizures it has been given intravenously and subcutaneously. Blackfan and Hamilton (38) found that, when given intravenously to children with acute glomerular nephritis, it not only checked convulsions, but reduced blood pressure and later promoted diuresis. To gain these effects they injected 20 cc. per kilogram of a 1 per cent solution of the salt at the rate of 10 cc. per minute. The author has employed the same method for the treatment of convulsions in adult nephritics with varying success. By the use of large enough doses of the salt, never less than 6 grams of $MgSO_4$, convulsions can usually be checked and blood pressure temporarily reduced, although there may be no lasting benefit. The cases to which it was applied by Blackfan and Hamilton presented edema. It is, however, useful in nonedematous subjects with convulsions. The author is inclined to ascribe the effects on both convulsions and blood pressure to the sedative action of magnesium, the diuresis to the acidifying action of the salt.

More recently Blackfan and McKhann (39) have reported favorable results in children from the use of magnesium sulfate by mouth or rectum, in most cases to prolong or maintain the beneficial action produced by intravenous treatment. By this means they have been able to prevent or delay recurrence of hypertension, convulsions and other cerebral manifestations. Weight losses indicate that the salt has a dehydrating effect. How far this is due to diuresis and how far to diarrhea the paper does not state, although the authors say that the diarrhea produced by the drug is small. The reduction of blood pressure and diminution of cerebral symptoms they are inclined to ascribe to relief of cerebral edema.

The author is inclined to believe that the action of magnesium sulfate given intravenously is quite different from that of the same drug given perorally and that cerebral edema may be a less universal cause of symptoms in acute nephritis than Blackfan and McKhann have indicated. In one child[7] in which intravenous magnesium sulfate had been efficacious, both hypertension and cerebral symptoms recurred while the drug was given by mouth. The patient, moreover, appeared dehydrated, and serum base was found to be low. Administration of saline and magnesium sulfate intravenously resulted in distinct improvement of clinical symptoms and blood pressure. Furthermore, intravenous hypertonic glucose solutions, which should be more rapidly efficacious than magnesium sulfate in relieving cerebral edema, seemed to have no beneficial effect in this case.

CALCIUM

Calcium will be considered later, in spite of its basic character, because of the close relation of its metabolism to that of phosphorus and to acid-base equilibrium.

CHLORIDE

The extreme variability of serum chloride in nephritis is well illustrated in figure 1. At first sight there seems to be no consistent connection between the chloride disturbances and the nature of the disease. Closer inspection, however, reveals a noticeable trend towards subnormal values in advanced renal disease and unusually high values in acute nephritis and the nephrotic syndrome. This distinction has been noted by most observers (21, 23, 25, 53, 89, 96, 101, 116, 123, 130, 131, 140, 150, 158, 163, 180, 181, 206, 207, 218, 228, 272, 301, 318, 329).

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[7] This case was under the care of Dr. Dan C. Darrow, who has kindly permitted it to be mentioned here.

The conditions which give rise to hypochloremia in patients with terminal nephritis have already been discussed in the section on sodium: vomiting, nausea and anorexia which cause direct loss of chloride or prevent replenishment of the chloride stores; excessive leakage of chloride through the kidneys in spite of serum chloride depletion; sometimes excessive fecal excretion of chloride. Of these causes of chloride deficiency de Wesselow (96, 97) recognizes only one, vomiting, which he considers a compensatory reaction to eliminate chloride, thus leaving base to combine with phosphate which is retained by the kidney. This hypothesis is invalidated by the fact, demonstrated by Salvesen (272) and by Peters, Wakeman, Eisenman and Lee (252) and others (217), that the vomitus of patients with advanced renal disease contains little or no free hydrochloric acid. For this reason vomiting eliminates sodium as well as chloride. In the kidneys also the normal ability to excrete sodium and chloride separately is impaired or abolished because of the incapacity to form and utilize ammonia for the neutralization of urinary acids (272). In consequence there is a more general tendency for chloride and sodium to move together in metabolism.

Continuous leakage of sodium chloride into the urine with or without vomiting creates a constant tendency to deplete both base and chloride stores of the body. This may not result in serum base or chloride deficiency because the tendency to regulate the water content of the body in accordance with the salt content is not lost. Salt deprivation causes more than the usual temporary diuresis and may lead to a serious grade of dehydration (251, 252).

The mechanism responsible for the production of hypochloremia is, then, readily comprehensible. It remains to explain the occurrence of hyperchloremia which, in figure 1, as in the studies of Salvesen (272) and others (25, 84, 251), is not uncommon. In the total 274 observations of serum Cl in figure 1, abnormally high values were found 74 times. Of these 29 were observed in patients, who, at the time presented a predominantly nephrotic picture and may, therefore, well be considered later with the nephrotic cases. In 18 instances the determinations were made on patients who had come to the outpatient clinic for examination or had just been admitted to the hospital. The hyperchloremia in these instances appears to be related to the high base observed under similar circumstances. In almost all cases free administration of fluids with production of an adequate urine volume brought the serum chloride rapidly to or below normal, often even when dietary salt was not restricted. With unlimited dietary salt and inadequate fluids chloride may, apparently, heap up in the blood because of the impaired concentrating ability of the kidneys. Peters, Wakeman, Eisenman and Lee (252) found that in spite of the tendency to continued leakage of chloride into the urine with low serum chloride levels, in advanced renal disease even the most vigorous administration of salt did not drive the chloride concentration in the urine above that of the serum.

If one examines the remainder of the cases in relation to symptoms and treatment they seem to fall into two groups: (*a*) patients who have been given large amounts of chloride to combat dehydration, base depletion and acidosis; (*b*) patients who, over long periods present high serum chloride, proportionally low bicarbonate, frequently with a base deficiency. A striking example belonging to the first class has been reported by Peters, Wakeman, Eisenman and Lee (251). The patient, an old man suffering from a urinary infection superimposed upon a chronic nephritis (probably arteriosclerotic), after the extraction of some teeth, vomited and refused fluids for some days. When first seen in medical consultation he was extremely dehydrated and overventilating, obviously from acidosis. Serum base was extremely low, 139.0 milliequivalents; bicarbonate greatly reduced, 2.5 milliequivalents, and chloride normal, 104.1 milliequivalents, in spite of the vomiting. He was immediately given, intravenously and subcutaneously, large quantities of normal saline and isotonic glucose solutions. Two days later he seemed somewhat improved, but still had evident acidosis. The serum base had risen to 148.5 milliequivalents, the lower normal limits; but bicarbonate only to 4.9 milliequivalents, while chloride had reached the extremely high value of 118.1 milliequivalents. Treatment with saline and glucose was continued, but sodium bicarbonate was added. Three days later dyspnea had disappeared and the old man was eating freely and feeling well. The serum electrolytes were practically normal: total base 154.0 milliequivalents, chloride 106.8 milliequivalents and bicarbonate 21.4 milliequivalents. The explanation of these peculiar disturbances seems to lie in the inability of the kidneys to form and excrete ammonia which forces them to utilize sodium and other fixed bases almost exclusively for the neutralization of acids. For this reason sodium in excess of chloride is continually escaping through the urine with other acids. If base is supplied only in the form of sodium chloride, the nephritic is not able, like the normal, to excrete the chloride ion while retaining the sodium. In behalf of the internal osmotic pressure and the water stores of the body he retains a certain amount of the sodium chloride as such without improving the bicarbonate deficit. In fact, the latter may increase in some cases because the bicarbonate is diluted by the retained chloride solution. The administration of bicarbonate under these circumstances, by providing an excess of available sodium, restores the normal electrolyte picture. This condition of hyperchloremia with bicarbonate deficiency has been spoken of as "chloride acidosis." This term, however, gives the erroneous impression that chloride, by accumulating in the blood, displaces bicarbonate. Had this been true in the case under discussion administration of further sodium chloride with the bicarbonate, when the serum chloride was already 118 milliequivalents, could not have bettered the serum electrolyte situation. At the time of the first observation the patient was extremely dehydrated, and the fluid deficiency had not been completely made up by the time of the second observation, as was indicated by a continuous fall of both blood oxygen capacity (15.5 to 12.3 to 9.4 volumes per cent) and serum proteins (6.8 to 6.0 to 5.6 per cent). Subsequently the bicarbonate was omitted and less salt given. Within six days base had fallen to 145.4 milliequivalents, and bicarbonate to 15.5 milliequivalents, while chloride had risen slightly, to 107.8 milliequivalents. At the same time a measure of dehydration had returned, for oxygen capacity had gone up to 11.0 volume per cent and serum protein to 5.9 per cent. These phenomena illustrate beautifully the balance maintained among a group of related functions and the concession that each must make to preserve the other from extinction. In the first instance there was a complete disorganization of the electrolyte and water equilibrium and metabolism. The water stores of the body and the blood were depleted, the base stores even more impoverished. There was far too little base to provide the usual concentration of bicarbonate even for the contracted fluid volume. Although the chloride concentration in the serum and extracellular fluids was still normal, the actual quantity of chloride was greatly reduced. When water and sodium chloride were given the latter was utilized not only to replace the sodium chloride which had been lost, but also as a somewhat unsatisfactory substitute for the missing bicarbonate, to restore base concentration and osmotic pressure and to permit the retention of further water. Had water enough been retained to reduce the chloride concentration to the normal level again, both base and bicarbonate would have been further reduced. However, if chloride had entirely replaced the bicarbonate deficiency and the water stores had been completely replenished, bicarbonate would have been driven even nearer extinction and chloride would have reached a still more abnormal level. Instead both base and water remained somewhat below the normal level. Only after the administration of bicarbonate furnished the lacking component was a completely normal electrolyte and water picture secured.

It is not improbable that the mechanism of chronic hyperchloremia with alkali deficit is somewhat similar. It is usually encountered in patients with advanced renal disease who are receiving unlimited sodium chloride. It is more likely to appear if the fluid intake is restricted. The hyperchloremia is more often associated with base deficit than with base excess. If it were due to specific retention of the chloride ion with displacement of bicarbonate, restriction of sodium chloride might be expected to rectify the electrolyte balance. As a matter of fact, limitation of sodium chloride, especially if the fluid intake is increased, usually results only in simultaneous diminution of chloride and base without any benefit to depleted bicarbonate. The latter can only be restored by giving bicarbonate.

These patients with hyperchloremia, especially those with the acute forms, probably account for a certain proportion of the cases with so-called "dry chloride retention" which have been described by Widal (329), Blum (50, 51, 52) and others (15, 16, 17, 175, 311). Such patients are reputed to retain large amounts of administered chloride without comparable quantities of water, and to excrete equally large quantities of chloride without proportional amounts of water when dietary salt is restricted (15, 16, 329). It has been frequently stated that the chloride thus retained was stored in high concentration outside the blood stream (50, 51). In terminal stages of nephritis, as in nephrosis and all other physiological and pathological conditions which have been investigated, the concentration of chloride in interstitial fluids and transudates exceeds that in serum. The discrepancies can, however, be ascribed to the differences in protein content and other physical characteristics of the two fluids (126,133, 144, 194, 229) and do not indicate lack of osmotic equilibrium as certain investigators have suggested. Any abnormal chloride accumulation must, therefore, be sought in tissue cells. Blum and van Caulaert (50, 51) claim that they have discovered accumulations of quite extraordinary magnitude in the brain, heart, liver, kidneys, and skeletal muscles of patients who have died of nephritis. What has been said above concerning similar accumulations of sodium demonstrated by the same authors, holds almost as well for chloride, although the existence of organic chloride combinations is perhaps more possible than similar combinations of sodium. van Caulaert (313) also reported high chlorides in the tissues of dogs after ablation of renal function, a finding which Atchley and Benedict (22) were unable to confirm. In this condition both sets of observers agree that serum chloride falls. In a case with urinary obstruction, also, high tissue chlorides with low serum Cl have been reported by Thiers (311). Ambard, Beaujard and Schmid (15, 16), without tissue analyses, believe in dry chloride retention and advance the theory that the chloride is combined with protein (17). It seems more than unlikely that such combinations can occur in media with reactions on the alkaline side of the isoelectric points of the proteins. The author in an extensive investigation of chloride balances in nephritis has failed to find any discrepancies large enough to force him to accept the theory of "dry chloride retention." If account is taken of extrarenal excretion in sweat, vomitus and feces, of changes in the serum which are reflected throughout a volume of extracellular fluid of uncertain magnitude and possibly also in part of the intracellular fluids, such a hypothesis becomes less necessary than it does if excretion is judged only by urine examination.

The concentration of chloride in normal serum is approximately twice as great as that in blood cells. In nephritis a more even distribution is not uncommonly seen and this fact has also been advanced in support of the dry chloride retention theory (47, 48, 175, 310). That chloride passes into cells from plasma when blood is acidified has been recognized since the experiments of Hamburger (138). It is not improbable that similar ionic shifts take place between tissue cells and adjoining intracellular fluids, although the permeability of tissue cells to chloride and the distribution of this ion in the body are too little known to allow certainty on this point. The reasons for these transfers of Cl have been carefully examined by numerous investigators and have been well defined by Van Slyke (314, 316). There is no reason for believing that the abnormal distribution of chloride between blood cells and serum in nephritis is more than an expression of acidosis. This is born out in those instances in which simultaneous bicarbonate or pH determinations have been reported.

Laudat and Grandsire (180, 181) have placed emphasis on the ratio of chloride to sodium in serum in Bright's disease. The use of such a ratio is subject to the general criticisms which apply to all ratios which compare independent variables. A high ratio may result from conditions which either increase chloride or diminish sodium. It is obvious that these two conditions are entirely unrelated, and can be connected by no trick of mathematics.

In general the subject with advanced renal insufficiency tends to waste chloride with its associated sodium both through renal and extrarenal channels, and with this sodium chloride a certain amount of body water. The condition is essentially dehydrating. Restriction of dietary chloride under such circumstances, especially with high fluids, leads to salt and water depletion. Administration of chloride will permit replenishment of the body's salt and water stores. Often enough it may increase urine volume. Partly this diuresis is due merely to restoration of the desire and ability to drink larger amounts of water. Sometimes the urine volume rises without proportional increase in the fluid ingested, and without loss of body weight (2, 244), suggesting that fluid is diverted from other excretory channels to the kidneys. It may be necessary because of vomiting or nausea to give the sodium chloride subcutaneously or intravenously. However, in certain cases the authors have found that small doses of ice cold salt solution (50 cc. of 2 per cent NaCl) given by mouth are well tolerated and may even allay nausea and check vomiting. Their effects may depend only upon the fact that, by restoring the desire for fluids, they remove the sense of distaste, which is enough to evoke an emetic response from an unsettled, irritable stomach. The administration of salt solution without an adequate amount of water may produce hyperchloremia because the ability to concentrate chloride in the urine is limited. Normal salt solution contains 0.9 per cent sodium chloride, while serum contains only 0.6 per cent. Normal saline alone, therefore, contributes sodium chloride in excess of water to the body fluids. Furthermore water is continually expended without salt by the lungs and skin. Therefore, it is imperative to administer water with the saline. This can be effected by the parenteral administration of glucose solution, if oral administration is impossible. Hyperchloremia does not necessarily constitute a contraindication to the use of salt. If it is associated with bicarbonate and base deficiencies it can be better combated by the administration of sodium bicarbonate. It must be borne in mind that the production of water diuresis will increase excretion of sodium chloride. Therefore high fluid intake should be covered by provision of a proportional quantity of salt.

The observation that hypochloremia is so often associated with extreme azotemia and that the blood non-protein nitrogen often falls after administration of sodium chloride has led Blum and others (52, 186, 312, 313) to speak of azotemia from lack of salt. They are inclined to refer the non-protein nitrogen accumulations directly to the hypochloremia and in support of this view have called attention to the rises of non-protein nitrogen which so commonly occur with dehydration and salt depletion in diabetic acidosis, pyloric obstruction and profuse vomiting from other causes (254, 312). To look upon the serum chloride deficiency as the direct cause of azotemia in these conditions seems hardly warranted. In pyloric obstruction administration of glucose solution, although not as effective as saline, at least mitigates nitrogen retention (250), which appears to be a product of oliguria and the increased nitrogen destruction of starvation, possibly aggravated by toxic destruction of nitrogen associated with dehydration. The azotemia of diabetic acidosis can probably be ascribed to similar factors. Inasmuch as hypochloremia in nephritis is also associated with dehydration and, if there is vomiting, some measure of starvation, and since sodium chloride combats dehydration and increases urine volume, Blum's findings are not surprising and do not necessarily indicate that the chloride deficiency is the cause of the nitrogen accumulations.

In the nephrotic syndrome serum chloride is usually increased, especially during edematous periods (23, 25, 53, 90, 101, 123, 158, 180, 181, 207, 212, 228, 253, 272, 301, 329). This is not, however, an invariable rule. In a certain proportion of cases Cl remains normal throughout the disease, usually with some elevation of bicarbonate (58, 85, 96, 253). Hypochloremia is rare and usually referable to some complicating condition or the development of renal insufficiency. Of the 11 observations in figure 1 in which Cl is below 98 milliequivalents, all were observed during fatal complications: four during terminal peritonitis in 3 subjects; one in conjunction with severe enteritis and diarrhea in a patient in the last stages of tuberculosis with amyloid disease; one during terminal peritonitis and sepsis in another case with amyloid disease complicating pelvic osteomyelitis with a vesical fistula; one in the last days of life of an elderly patient with a peculiar nephrosis associated with profound anemia, multiple arthritis, heart failure, diarrhea and vomiting of unknown origin.

It has been suggested that the increases of chloride or bicarbonate in the serum merely serve to combine with the base which has been freed by reduction of the serum proteins. Ordinarily the proteins of the serum neutralize 15 to 20 milliequivalents of base. In the extreme hypoproteinemia of nephrosis only 5 to 10 milliequivalents of base may be combined with protein, leaving 10 or 15 which must find other acids, unless the base is to fall. If this is the proper explanation of the bicarbonate and chloride increases, the latter fall short of their purpose because, as has already been pointed out, serum base deficiency is extremely common in the nephrotic syndrome. If chloride or bicarbonate or both tend to replace protein in combination with base, the sum of the combining equivalents of bicarbonate plus chloride should be abnormally large in nephrosis and should vary inversely as the concentration of base combined with protein. Although bicarbonate plus chloride lay above the upper normal 133 milliequivalents 33 times in the series of figure 1 and below the lower normal limit, 125 milliequivalents, only 3 times, no correlation could be discovered between this sum and the serum protein concentration. Moreover, at all levels of protein the sum of bicarbonate and chloride usually lay within normal limits.

Another indication that the excess of base or bicarbonate or both does not serve to neutralize base released by proteins or other acids is the close reciprocal relation exhibited by bicarbonate and chloride. This is illustrated by figure 2, constructed from the author's data.

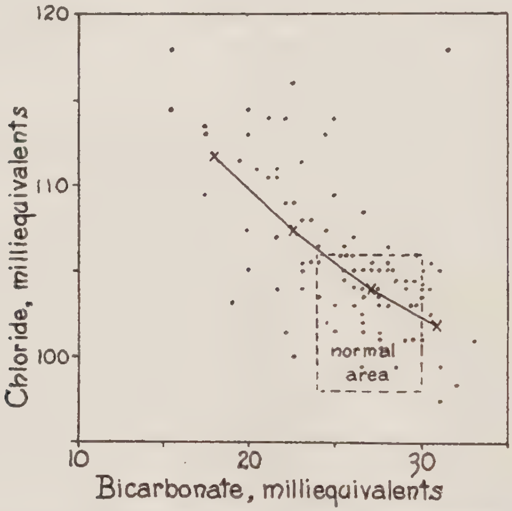

Fig. 2. The Relation of Serum Chloride to Serum Bicarbonate in Patients with the Nephrotic Syndrome

The crosses represent group averages and the line connecting them indicates the trend of the relation between the two functions. In computing the averages for the construction of this line the single point in the extreme upper right hand corner of the figure was omitted.

Certain cases have a tendency to exhibit consistently, throughout the edematous stages of the disease, high serum chloride, usually with a moderate degree of bicarbonate deficiency. Others maintain bicarbonate constantly near or slightly above the upper normal limit. In the latter serum chloride is usually normal. The slope of the line in figure 2, describing the average relation of bicarbonate to chloride is, at normal or high levels of bicarbonate, at least, somewhat less than the 45° to be expected if chloride were displaced by bicarbonate in equivalent amounts. In nephrosis serum chloride tends to be absolutely high or high in proportion to bicarbonate.

This tendency for chloride and bicarbonate to play reciprocal rôles has been frequently noted, as has the tendency for some patients with edematous nephritis to have normal and others high serum chlorides. In fact it is probably the latter point that has in the past given rise to some controversy concerning the typical electrolyte picture in nephritic edema among investigators who have not made extensive investigations. The two electrolyte pictures are not associated with any characteristic clinical differences. Although it is possible, by the use of alkalinizing or acidifying measures, such as the administration of sodium carbonate or ammonium chloride to modify the electrolyte picture, the latter resumes its characteristic abnormality after the treatment is discontinued (10, 123, 244, 253). Laudat and Grandsire (180, 181) in 2 cases found that serum Cl fell without a comparable change of base when dietary salt was restricted. This is not invariably the case. Even when salt restriction is followed by some diuresis the proportions of base, chloride and bicarbonate in the serum may remain comparatively unaltered (212, 244, 253, 317). On the other hand, with clinical improvement and disappearance of edema the electrolyte picture becomes normal whether bicarbonate or chloride was previously high (58, 244, 253, 317).

The relation of chloride and sodium to edema has been discussed at some length in the section on sodium above, where it was also mentioned that in nephritic edema, while the proteins were low, a diuretic response after acidifying chlorides often failed to appear or could be provoked only by the production of an unusual degree of acidosis. In one of the author's cases (253) serum chloride was driven up to 124.2 and bicarbonate down to 4.3 milliequivalents without production of important diuresis.

The tendency to sustained hyperchloremia without incontrovertible evidence that there is any peculiar impermeability of the kidney to the chloride ion has given rise to the idea that the abnormal chloride increment may exist in some peculiar form, possibly in non ionized organic combinations that renders it unavailable for excretion. Hanke and Donovan (139) claim that lipoid combinations with chloride can be found in the gastric mucosa and other tissues. Sunderman (309)

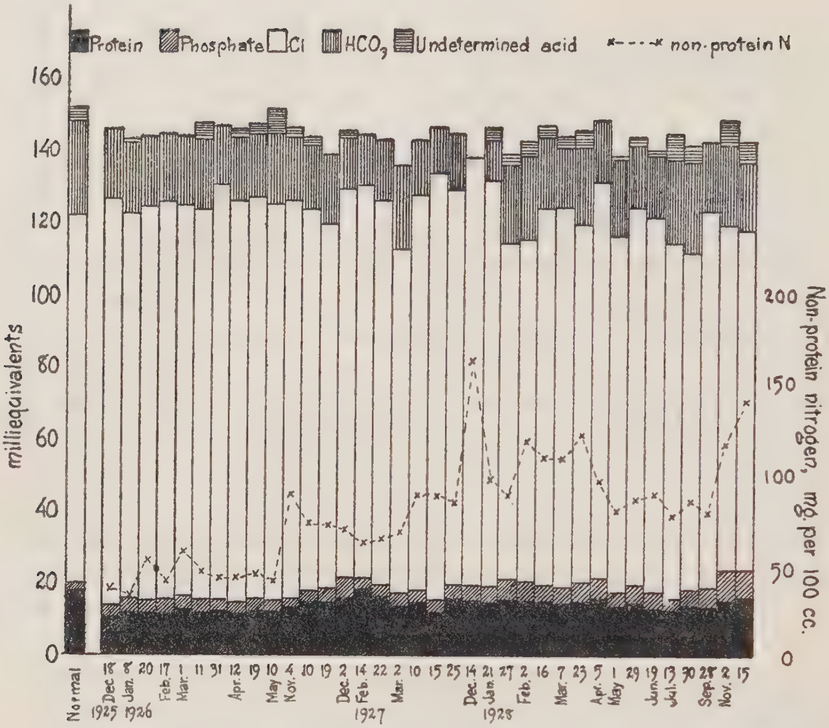

FIG. 3. ELECTROLYTE STUDIES THROUGHOUT THE COURSE OF A CASE OF GLOMERULONEPHRITIS OF THREE YEARS' DURATION

In the first column the average normal electrolyte pattern of human serum is given for comparison. Total base is not separately indicated, because it is equal to the sum of the acids. "Undetermined acid" represents the difference between total base and the acids which were determined by analysis: proteins, bicarbonate, chloride and phosphate. It is composed of sulfate and organic acids. Where undetermined acid is not given total base was either not determined or was practically equal to the sum of the determined acids.

The early stages of the disease illustrate the picture of acute nephritis, predominantly of the nephrotic type, with low proteins, high chloride and slight reduction of bicarbonate. As evidence of kidney scarring and renal insufficiency develop, while edema disappears and albuminuria diminishes, the proteins rise to a slightly higher level and the other electrolytes become more variable, with a tendency for chlorides especially to fluctuate, reaching both extremely high and low levels as the clinical symptoms vary.

In the terminal stages phosphate and undetermined acids increase, while chloride remains low.

Bicarbonate deficiency persists throughout the disease, but in this case never becomes extreme. The variability of electrolyte concentrations and patterns in relation to symptoms is quite evident.

During the early nephrotic period, which includes the first two hospital admissions, December 18, 1925, to January 20, 1926, and February 17 to May, 1926, edema recurred whenever salt restriction was interrupted. Limitation of salt did not, however, result in any important reduction of serum chloride or base (compare the admission examinations, December 18 and February 17 with subsequent determinations). During this stage of the disease the blood pressure was usually normal and blood non-protein nitrogen was but little elevated.

discovered that in serum of certain individuals, after it had been dried, all the chloride could be recovered only if the dried serum was leached for a long time with water before analysis. He found that chlorides mixed with fat emulsions behaved in the same manner. The author has frequently found in highly lipemic sera from diabetic or nephritic subjects, in determining the total electrolyte balance, values for total base that were too low to neutralize all the acid radicals. The discrepancies were too large to be ascribed to faults in the factors employed for the calculation of base combined with protein. Peters and Eisenman (248) have been unable to demonstrate chlorides in lipoids secured by extracting bloods with fat solvents which will not dissolve chloride. When dried serum is extracted directly with petroleum ether, which will not dissolve chloride, no chloride is found in the petroleum ether extracts. On the other hand, if certain highly lipemic sera are first extracted with hot alcohol and ether by the method of Bloor, chloride can be extracted from the dry residue of the alcohol ether extract with petroleum ether. Chlorides have not been extracted by this technic from normal or most pathologic sera. This comes far from constituting satisfactory evidence of lipoid-chloride combinations in serum, and there is much evidence against their existence, especially the complete diffusibility of serum chloride and the satisfactory chloride equilibria which have been demonstrated in nephritic edema between sera and transudates which are almost free from lipoids.

If, in the course of the nephrotic syndrome, renal insufficiency develops,—e.g., in progressive chronic glomerular nephritis,—the electrolyte pattern may change and the serum chlorides may fall to subnormal

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On subsequent admissions dietary salt restriction regularly gave rise to hypochloremia, with simultaneous base deficiency. This is illustrated by the rapid fall of Cl and base between November 4 and 19, 1926, February 14 to March 2, 1927, and January 21 and 27, 1928. The restoration of a more normal electrolyte picture by the administration of sodium chloride is shown in the determinations of December 2, 1926, March 10, 1927, and February 16, 1928.

The extremely high chloride of December 14, 1927, discovered when the patient came to the dispensary for observation, followed a period in which fluids had been restricted because of nausea, without vomiting. Unfortunately total base and bicarbonate were not determined on this occasion. However, unless bicarbonate was lower than at other times in the course of her illness, base must have reached or exceeded the upper normal limit on this occasion.

In the terminal period the multiplicity of symptoms, including heart failure and vomiting, makes analysis of the electrolyte data almost impossible.

There is little detectable relation between non-protein nitrogen and any electrolyte components.

levels (244). Usually such a change is associated with loss of edema and complete transformation of the clinical picture. The progressive changes in such a case are illustrated in figure 3. In the nephrotic stage of glomerular nephritis an acute exacerbation of the disease may produce an apparently similar change, even without disappearance of the edema. This is illustrated by figure 4, from one of the author's cases. In this patient the sudden reduction of serum base and chloride that developed with the appearance of the acute nephritis were attended by no diuresis; in fact edema increased. At the time of the first observation the patient presented a simple nephrotic picture with general anasarca. Under salt restriction and a generous diet, supplemented by administration of urea, he gradually lost weight in spite of the retention of considerable nitrogen, which presumably was utilized for the construction of tissue proteins, because blood non-protein nitrogen did not rise proportionally. With the loss of weight there was a large negative chloride balance. The hyperchloremia, however, did not diminish. On the day of the second observation, May 26, he developed a respiratory infection accompanied by fever and some hematuria. The diuresis ceased and the chloride excretion diminished. Between May 26 and June 2 there was almost exact chloride equilibrium, which is mirrored in the constancy of serum chloride. After June 2 the patient's condition became much worse. Urine volume diminished, hematuria increased, blood pressure rose, weight increased in spite of extreme anorexia, nausea and vomiting; he became more and more stuporous and finally had a series of convulsions. Until June 24 salt restriction was continued. Metabolism studies during this period were not entirely satisfactory because of the mental condition and occasional vomiting. The reduction of serum chlorides between June 2 and 20 appears, however, to have been due less to vomiting than to retention of water without salt. Base and chloride fell together in amost exactly equivalent amounts. To interpret this as an indication that water is especially retained by the kidney, that there is displacement of chlorides to the tissues or wastage of chlorides through extrarenal channels is unwarranted. The changes in the serum are those one would expect if, because of the oliguria, all urinary components were equally retained. In the absence of any supply of sodium chloride, serum chloride and base were reduced by dilution. During the next

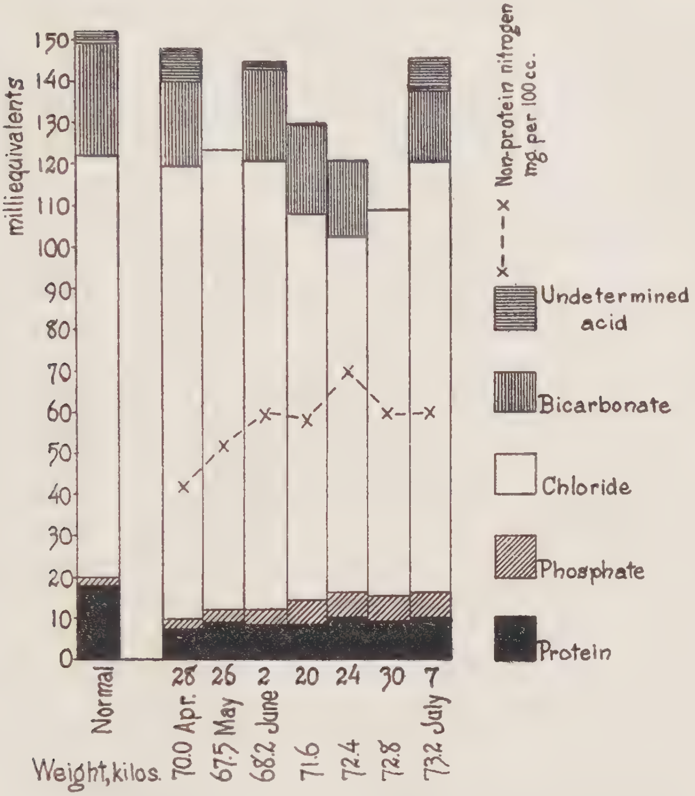

Fig. 4. Serum Electrolytes in a Case of Glomerulonephritis

In the first column the average normal electrolyte pattern is shown for comparison. Total base is not separately indicated because it is equal to the sum of the acids. "Undetermined acid" represents the difference between total base and the acids which were determined by analysis: protein, bicarbonate, chloride and phosphate. It is composed of sulfate and organic acids. June 20 the "undetermined acid" was too small (only 0.4 m.eq.) to show. On June 24 total base was not determined. On May 26 and June 30 neither total base nor bicarbonate was determined.

At the time of the first examination, April 28, the clinical picture was predominantly that of the nephrotic syndrome. With salt restriction, high protein high caloric diet and urea administration the weight fell gradually and the chloride balance was negative up to May 26. On this day he developed an acute respiratory infection, followed shortly by hematuria and increased blood pressure. Diuresis ceased and he came into chloride equilibrium. After June 2 he rapidly became worse; vomiting and anorexia set in and he finally had a series of convulsions. Between June 2 and 24 the weight increased. The chloride balance may have been slightly positive, although the mental condition and vomiting prevented exact metabolism studies. Up to this time salt restriction was continued. From June 24 to 30 a positive chloride balance was secured by the daily administration of 5 grams of sodium chloride. After this a slight positive chloride balance was maintained, although salt restriction had been resumed. Between June 24 and 30 the clinical condition improved greatly.

four days, June 20 to 24, the same process continued. After this 5 grams of sodium chloride were given daily. This produced a considerable positive chloride balance, causing serum chloride to rise. It is worthy of note that urinary chlorides increased little during this period, nevertheless edema was not aggravated. For a time, indeed, the urine volume rose and the weight fell; the general condition was greatly improved. After June 30 a salt poor diet, containing about 2 grams of sodium chloride daily, was resumed. On this diet enough salt was retained to restore serum chloride to normal, again without a proportional amount of water.

The case illustrates several points. It suggests what Fishberg (114, 116) has called "the unitary nature of impairment of renal function." The selective nature of renal insufficiency may be more apparent than real because its effects are so much influenced by extraneous and intrinsic modifications of metabolism. In the extreme condition of anuria there can be no doubt that urine as a whole is retained and all products, including water, which are usually excreted in the urine, must accumulate in the body, except in so far as failure of the kidneys may stimulate unusual activity on the part of other excretory organs. Between anuria and lesser grades of urinary restriction there must be a gradual transition of functional disturbances. This limitation of urine volume as a whole must be distinguished from renal insufficiency in the usual sense. There is, in this case, none of that tendency to waste salt which accompanies hyposthenuria. With salt restriction salt is retained to meet a salt deficiency which, in this case is provoked by increase of body water. Under these circumstances additional salt was not excreted nor did it promote further water retention. It was merely utilized to restore the normal electrolyte pattern in the serum and body fluids. How far chloride would have risen before further water retention developed is uncertain, as the patient refused to remain longer in the hospital. He seemed to be resuming the earlier nephrotic characteristics.

The sudden change of salt and water excretion that began with the acute nephritis suggests the introduction of some new hydropigenous influence. Possibly, as Volhard (320) has suggested, in acute nephritis circulatory and inflammatory changes in the kidneys interfere in a more or less mechanical way with the excretion of both water and solutes. Such an interpretation is not, however, obligatory. If the retention of water were referable entirely to renal impermeability, blood dilution might reasonably be expected. During the acute nephritis attack in this case, however, the serum proteins increased, although the diet was extremely reduced. Both albumin and globulin fractions were affected. This suggests, although it does not prove, that the blood became more concentrated, presumably by loss of fluid to the transudates. The increase of oliguria may, therefore, have been due less to renal impermeability than to exaggeration of the tendency to transudation. Circulatory and vascular changes may have furthered the effects of serum protein deficiency in driving fluid from the blood stream.

In acute nephritis serum chloride is extremely variable, corresponding, to a certain extent, with the variability of the clinical picture and symptoms (21, 25, 116, 140, 163, 301, 308). High chloride is more common in those patients with the greatest tendency to edema, low chloride in those with extreme azotemia and renal insufficiency. Hartmann (140) is inclined to ascribe the electrolyte disturbances entirely to symptoms, such as vomiting. With recovery from the nephritis the chlorides return to normal. If the disease progresses the whole electrolyte picture develops the pattern characteristic either of the nephrotic syndrome or of renal insufficiency, according to the form which the nephritis assumes.

Dietary sodium chloride restriction has been recommended and employed quite indiscriminately for the treatment of renal diseases. It has been claimed that it reduces blood pressure (14, 15, 16, 28), albuminuria (15, 16, 329) and relieves headaches and central nervous system symptoms (15, 16). Its value in the treatment of edema is unquestioned and follows quite logically from what has been said of base and chloride metabolism in the disease. Its effects on albuminuria and hypertension (34, 116, 225, 235) have not been established and it has been pointed out that hypochloremia is by some held responsible for some of the symptoms of uremia. As a means of reducing polyuria of renal insufficiency (16, 329) salt restriction must be used with caution because this polyuria is apparently a protective reaction. From what has been said above it should be clear that the regulation of the sodium chloride intake is not a matter for routine prescription, but one that requires the most critical consideration in each individual case.

BICARBONATE, pH AND ACID-BASE EQUILIBRIUM

Bicarbonate may be considered as the vehicle by which the organism is enabled to carry a reserve of base in a form which makes it readily available for the neutralization of any other acids which may find access to the body. It is merely a combination of alkali with carbon dioxide, a weak acid of which a continuous supply is provided by the processes of metabolism in all tissues and which differs from other acids in the fact that it is volatile and can be excreted by the lungs. For these reasons bicarbonate has been called the alkali reserve and becomes a measure of the amount of base not combined with, and therefore available for the neutralization of, other non-volatile acids. If more than the usual quantities of acids gain access to the body they displace bicarbonate from its combination by the reaction,

$$HA + BHCO_3 \rightleftharpoons BA + H_2O + \overset{\text{excreted by lungs}}{\overset{\uparrow}{CO_2}}$$

The CO_2 is excreted by the lungs. On the other hand if, as in pyloric obstruction, acid is lost without an equivalent amount of base, the base which is released by loss of the acid immediately combines with carbon dioxide to form bicarbonate. The reactions involved may be suggested by the following equilibrium equations:

$$(1) \qquad NaCl + H_2O \rightleftharpoons NaOH + \underset{\text{excreted by stomach}}{\underset{\downarrow}{HCl}}$$

$$(2) \qquad NaOH + CO_2 \rightleftharpoons NaHCO_3$$

It is evident that bicarbonate is reduced by accessions of acid or losses of base; increased by losses of acid or additions of base.

Changes of bicarbonate are attended by disorganization of the constitution of the tissues. For this reason and because the amount of base combined with or available for combination with bicarbonate is limited, these changes serve only as a temporary expedient for the resistance of acidifying or alkalinizing disorders. The chief or permanent defense resides in the kidneys which select for excretion in the urine the ions which are present in the body in excess, retaining those of which there is a deficiency. The principle reactions by which acid ions are excreted without equivalent amounts of base are: (*a*) substitution for fixed base of ammonia, which is formed from the neutral substance urea; (*b*) the formation of a highly acid urine from practically neutral blood serum, which permits weak buffer acids to be excreted with far less base than they held in the body.

The great weight of evidence indicates that the formation of ammonia from urea is a function of the kidneys (9, 32, 56, 118, 258, 261) and is demonstrably impaired in renal insufficiency (200, 236, 259, 272, 315). In the end products of normal metabolism there is a certain excess of acids over bases, owing chiefly to the fact that neutral sulfur and phosphate in organic compounds, mainly protein, are converted to inorganic sulfates and phosphates. Normal urine is, therefore, more acid than blood and, besides, contains a certain amount of ammonia. The titratable acidity (that is the quantity of acid which reduces the pH of the urine below that of the blood) plus the ammonia represents acid excreted without the base which it held in the body. In normal subjects Van Slyke, Linder, Hiller, Leiter and McIntosh (315) found the ratio of ammonia to titratable acid 1.0 or greater. In renal insufficiency this ratio diminished steadily as ammonia formation decreased. Salvesen (272) Linder (189) and others have shown that the administration of hydrochloric acid which, in the normal subject, greatly augments urinary ammonia, in the advanced nephritic has no such effect. In renal insufficiency, then, one of the chief defences against base depletion is injured; the body is forced to depend entirely upon its ability to excrete an acid urine. This is, however, useful only in the excretion of weak buffer acids such as phosphate and organic acids. Even in the most highly acid urines such strong acids as hydrochloric and sulfuric must be completely neutralized and therefore carry with them equivalent amounts of base. The result of this incapacity is a continuous tendency to deplete the base stores of the body, in the course of which serum sodium suffers. The chief factor which makes alkali deficit the commonest electrolyte disturbance of advanced nephritis is, therefore, serum base deficiency (23, 140, 251, 272). To what extent other bases may be called upon to aid sodium is uncertain. Serum potassium is usually unaltered or slightly increased. Excessive excretion of potassium has been demonstrated in some cases (59, 187), but this may be merely the result of tissue wastage. Calcium also seems to be sacrificed. From the standpoint of electrolyte equilibria, however, the sodium changes have been given especial attention, perhaps because they are so easily detected.

A further cause of serum bicarbonate deficiency is displacement by phosphate and sulfate. With the discovery of serum phosphate increases in nephritis by Greenwald (134), Marriott and Howland (209) and others, there was, at first, a tendency to refer the acidosis of nephritis directly to retention of phosphates by the kidney. Subsequent work has shown that hyperphosphatemia is only a minor contributory cause of nephritic alkali deficits (23, 60, 79, 112, 140, 206, 251, 272, 286). The quantitative importance of serum sulfate accumulations in reducing bicarbonate has been little studied. That serum inorganic sulfate may rise in advanced nephritis has been clearly established (23, 92, 93, 156, 195, 220, 323, 324). Atchley and Benedict (23) believe that reductions of serum base and increases of phosphate and sulfate together can account for the whole of the alkali deficits of advanced renal disease. Salvesen (272) and Peters, Wakeman, Eisenman and Lee (251), because they found such high concentrations of undetermined acids in the serum of some cases, concluded that organic acids also accumulated in the serum in the terminal stages of renal disease. Peters, Wakeman, Eisenman and Lee (251) suggested that these acids might be partly ketone bodies, products of vomiting and resultant starvation. Atchley and Benedict (24) deny the incidence of excess organic acids in the blood and of ketosis in nephritis. Their own data show, however, in the blood of at least one patient, as much as 14 milliequivalents of acid other than protein, bicarbonate, chloride, phosphate and sulfate, which is presumably organic in nature. Their failure to demonstrate ketosis in patients who were vomiting is of no significance unless the vomiting was sufficiently frequent to cause carbohydrate starvation. If, with vomiting of this severity, which is not uncommon in nephritis, ketosis did not develop, one would be forced to conclude that nephritis bestows on its victim some especial power to burn fat without carbohydrate. What is known of the subject, on the other hand, only indicates that the subject with severe renal insufficiency is more than usually susceptible to ketone acidosis because he is less able to eliminate the ketone bodies in the urine (78). Jervell (155) has demonstrated unusually large amounts of lactic acid in the blood of certain nephritic patients. The lactic acid excess may be referable to heart failure or anemia or both.

Van Slyke, Linder, Hiller, Leiter and McIntosh (315) found that the ammonia: titratable acid ratio in the urine rose again when renal insufficiency became extreme. This was due not to resumption of ammonia formation, but to limitation of the ability to excrete an acid urine. Others have demonstrated a tendency to fixation of urinary acidity, comparable to the fixation of specific gravity discussed earlier (182, 275, 276). The reaction and the acid content of the urine are products of so many factors that it is impossible to consider them as a single function. Fixation of acid excretion may imply no inability to separate base from buffer acids when the latter are excreted; it may only indicate inability to concentrate certain or all of these buffer acids in the urine. The behavior of phosphates and certain organic acids suggests that the capacity to excrete individual acid radicles is impaired.

In certain cases low bicarbonate may be associated with hypochloremia. It has been pointed out that the bicarbonate ion is usually not directly displaced by chloride; the condition is not in the most exact sense a chloride acidosis comparable to that which can be produced by the administration of hydrochloric acid or acidifying salts. The mechanism of its production and the conditions under which it appears have been discussed at length in the sections on base and chloride.

Muntwyler, Way and Binns (228) have reported high serum bicarbonate and pH in a group of patients with hypertension and arteriosclerosis with little or no evidence of renal insufficiency. Spontaneous alkali excess has also been observed occasionally in patients with glomerular nephritis. No studies which aid in the explanation of such alkalosis have been made. Of more importance is the tendency for patients with renal insufficiency to develop alkali excess after the administration of bicarbonate and the reported inability of such patients to excrete administered bicarbonate and alkaline urine. The latter characteristic seems quite incompatible with the known tendency of these subjects to waste base in the urine. Gamble (122), Marshall (210), Mainzer (203) and others have shown that bicarbonate in the urine, as in the blood, represents only base not combined with other acids, which has seized carbon dioxide that is present in the urine as in all body fluids. Urinary bicarbonate serves as a means for the excretion of any excess of base which may accumulate in the body without the acid with which it was combined in the organism. Bicarbonate excretion, therefore, plays a rôle in the selective excretion of base similar to that played by ammonia in the selective excretion of acid. Palmer, Van Slyke, Salvesen and Jackson (237, 238) found that bicarbonate administered to normal individuals produced a calculable increase of serum bicarbonate which was proportional to the amount of salt given, and that the urine became alkaline when serum bicarbonate rose above 70 volumes per cent. In nephritic subjects the urine frequently remained acid after serum bicarbonate had risen above this critical level. One can not infer from this fact that failure of the urine to become alkaline is proof of inability of the kidneys to excrete sodium or other bases.

In pyloric obstruction Gamble and Ross (124) and Hartmann and Smyth (141) have observed acid urine when the bicarbonate and the pH of blood were both far above the normal limits. Under these circumstances the administration of sodium chloride resulted in the simultaneous alkalinization of the urine and reduction of the bicarbonate and the pH of the blood. Acid urine with blood alkali excess seems to occur when the total content of base in the body is depleted, and indicates the unwillingness of the organism in behalf of acid-base equilibrium to tolerate further inroads on osmotic pressure and body water. When, by the administration of sodium chloride, serum base and sodium chloride are restored, acid-base equilibrium is readjusted by the elimination of the excess alkali through the kidneys as bicarbonate. In nephritis base deficiencies of considerable degree are common, especially in the presence of alkali deficits. The failure to excrete administered bicarbonate when serum bicarbonate reaches the critical level may be referable only to this total base deficit and may be quite comparable to the analogous situation in pyloric obstruction.

The failure of urinary pH to rise after bicarbonate to the levels which can be obtained in normal individuals may depend on different factors and may indicate an inability to concentrate sodium to the normal extent, even though the total excretion of base remains unimpaired. This may be only another example of the general loss of concentrating ability with hyposthenuria. It follows from both these phenomena that it may be somewhat easier, as Ellis (104) and others have suggested, to produce alkali excess and its associated symptoms, including tetany, in nephritic subjects than it is in normals. In any case acidosis of nephritis is not, like that of diabetic acidosis, associated with a consistent large increase of acid production. If one desires to combat the alkali deficiency with bicarbonate, only enough of this salt need be given to neutralize the excess of acid produced in normal metabolism.

Determination of bicarbonate tolerance has been advocated as a test of renal function. In most of the methods proposed 10 to 20 grams of sodium bicarbonate are given by mouth, after which the reaction of the urine is tested by colorimetric determination at intervals. Numerous investigators have shown that the reaction of the urine in nephritis remains unchanged or does not become as alkaline as that of normal subjects treated in the same manner (71, 204, 268, 293). For the reasons that have been stated above the test affords no accurate measure of either the alkali deficit or of the renal excretory power.

The indications for the therapeutic use of bicarbonate have been defined in preceding sections. The chief of these is reduction of total base at the expense of the serum bicarbonate fraction. The use of bicarbonate to combat alkali deficiency due to the accumulation of foreign acids is justifiable when dyspnea and acidosis are distressing symptoms, but can not effect any fundamental improvement of the condition; this can only come from increased elimination of the abnormal acid increments. Sansum, Blatherwick and Smith (274) advocate as a general therapeutic measure for the treatment of nephritis, the use of diets which yield an alkaline ash. Maintenance of an alkaline urine by means of such diets, they claim, improves hypertension, albuminuria and other symptoms. Lyon, Dunlap and Stewart (198) report striking alleviation of symptoms with reduction of azotemia by this treatment in a group of nephritics with renal insufficiency. The author has been unable to duplicate these results and can find no theoretical or empirical justification for the indiscriminate use of alkali or alkaline diets. Stieglitz (303) claims that alkalis irritate the kidneys and, if given over a period sufficiently long, may provoke a true nephrosis. Lashmet (177) ascribes to acid diets a special beneficial and diuretic influence. The diuretic effects of acidifying measures are well established, but constitute no general indication for the use of acid diets or acid diuretics. Both acidifying and alkalizing measures should be used, as they may be required, for the most part to restore the normal electrolyte content of the body, sometimes to distort the electrolyte pattern in order to provoke a diuretic response.

As in other states of primary alkali deficit and excess the pH of the serum undergoes comparable alterations, falling or rising with the bicarbonate concentration (150, 228, 230, 272). This is only one example of the general rule that the respiratory response to changes in the alkaline reserve is never so complete as to prevent all alterations of the pH, which is dependent upon the relative proportions of free carbon dioxide and bicarbonate in body fluids.

In the nephrotic syndrome Magnus-Levy (200) claims that there is a disturbance of the acid-base excretion which results in the production of an alkaline, highly ammoniacal urine. For reasons detailed above high urinary ammonia is commonly associated with increased acid elimination. In fact, alkaline, ammoniacal urine is usually accepted as evidence of bacterial decomposition. The same combination without urinary tract infection would indicate some anomaly of metabolism. It has been observed in certain cases without nephrosis,—for example, by the author, in a case of myelomatosis with extreme disturbance of calcium and phosphorus metabolism. Magnus-Levy (200) has also called attention to its association with disturbed phosphorus excretion. It does not consistently occur in nephrosis. In this condition, however, for reasons that have been discussed in the sections on total base and sodium, under certain circumstances, after acidifying measures, less sodium is excreted than usual and the extra acid in the urine is neutralized predominantly or exclusively by ammonia.

The tendency for certain patients with the nephrotic syndrome to maintain serum bicarbonate somewhat above the normal level, while others as consistently exhibit low bicarbonate, and the reciprocal relationships between bicarbonate and chloride in these patients have been discussed in the section on chloride.

The theory that ammonia, accumulating in the body, is responsible for the symptoms of uremia (120) has lost its adherents since improved analytical methods have proved that blood ammonia in nephritis is not significantly increased. Acidosis has also been exonerated from responsibility for most of the symptoms identified with uremia, although, by promoting dehydration and provoking dyspnea, it may contribute to the general clinical picture of terminal nephritis which is so loosely included under this term.

SULFATE

Increases of serum inorganic sulfate have been demonstrated in the terminal stages of nephritis by a number of observers (23, 59, 92, 93 156, 195, 220, 323, 324) and are partly responsible for the alkali deficits observed in this stage of the disease. The sulfate accumulations have been attributed to retention of this ion by the kidney, although studies of sulfate excretion to support such an inference are not available (59, 302). Most observers agree that this retention appears only in the terminal stages of the disease when the blood non-protein nitrogen is also elevated, although there is no definite relation between the degree of azotemia and the serum sulfate concentration. Wakefield, Power and Keith (324) have recently reported high serum sulfates in earlier or milder stages of nephritis, in some cases when blood urea and creatinine were still within normal limits, phenolsulfonephthalein excretion still unimpaired and urine concentrating powers little diminished. The differences between their results and previous observations Wakefield and his associates ascribe to greater accuracy of technique. Their method gives values in serum from patients without renal disease of 0.8 to 1.8 instead of the previously accepted limits of normal variation, 0.5 to 3 mgm. of sulfur per 100 cc. In advanced renal insufficiency they have found as much as 26.0 mgm. per 100 cc. This last figure would represent about 16 milliequivalents. Such figures are hard to accept without confirmation and without details of the analytical method employed. The data would indicate that sulfate is among the first inorganic ions to accumulate in the blood in nephritis and a far more important factor in the production of alkali deficits than even its most ardent advocates had supposed.

INORGANIC PHOSPHATE

Increases of serum phosphate in nephritis were noted in 2 cases with uremia by Strauss (304) in 1902; but their clinical importance was first emphasized by Greenwald (134) and Marriott and Howland (209). Since then hyperphosphatemia has been clearly recognized as one of the results of renal insufficiency and has been held responsible for acidosis, uremia and renal rickets. It usually appears in patients with advanced renal insufficiency and high blood non-protein nitrogen (23, 60, 77, 79, 93, 95, 96, 112, 140, 206, 272, 286, 289), but may occur in acute nephritis (60, 140, 244) and even in the chronic nephrotic syndrome (253). The frequency of increases in the various forms of the disease is shown in figure 1.

In advanced renal insufficiency high serum phosphate is seldom seen without a considerable degree of azotemia, but phosphate and non-protein nitrogen do not parallel one another with any accuracy. Boyd (60), Byrom and Kay (79), de Wesselow (95, 96), Schmitz, Rohdenberg and Meyers (286) and others consider that the serum phosphate concentration is an important criterion of prognosis in nephritis. It rather closely parallels creatinine, although the latter usually rises earlier in the disease (59, 286, 289). If the serum inorganic phosphorus exceeds 7 mgm. per 100 cc. the prognosis is very grave and if it exceeds 10 mgm. early death may be expected. Figure 5 shows the relation of serum inorganic phosphorus to blood non-protein nitrogen in patients of the author's series with advanced renal insufficiency. There is a general tendency for the two to increase together, in spite of considerable scattering and certain individual instances in which a rise in one component is attended by no change in the other. The agreement is more exact in chronic glomerular nephritis (points indicated by dots) than in either arteriosclerotic nephritis (indicated by crosses) or surgical renal conditions (circles). In chronic glomerular nephritis phosphate was only twice slightly above normal when non-protein nitrogen was below 40 mgm. per 100 cc. The 12 instances in which non-protein nitrogen was elevated, while phosphate remained normal deserve special attention. Six occurred during intercurrent attacks of acute nephritis or acute exacerbations of the chronic disease, on three occasions with the development

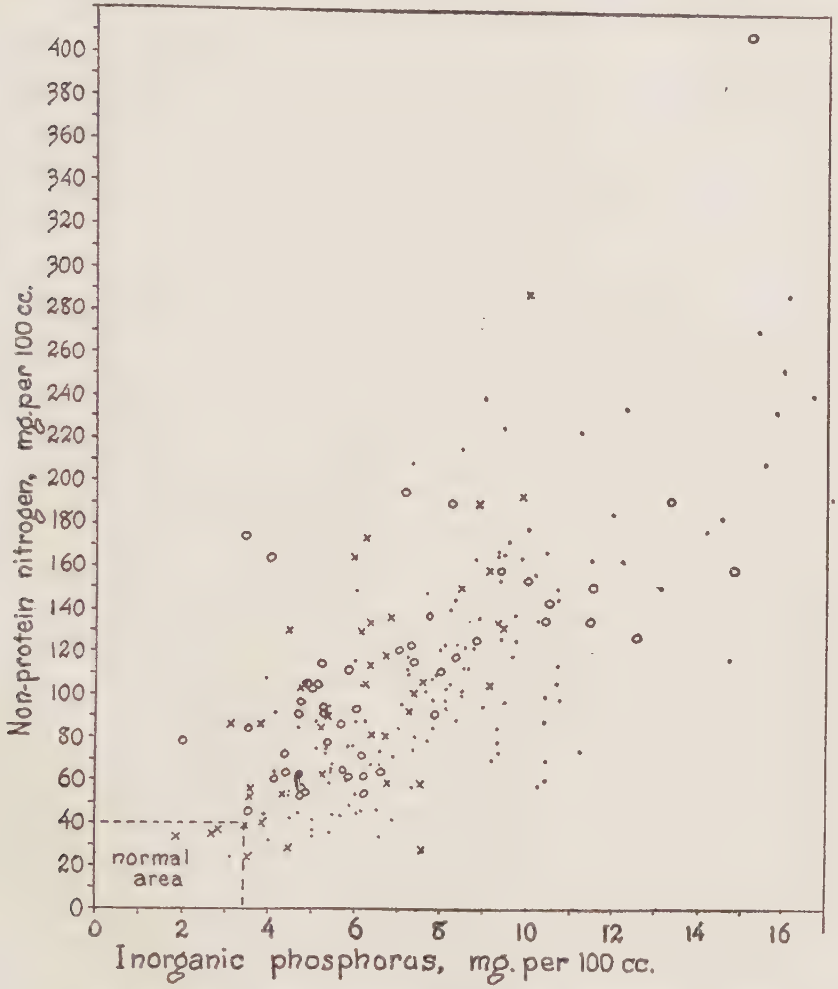

Fig. 5. The Relation of Serum Phosphate to Blood Non-protein Nitrogen in Renal Insufficiency

Dots represent chronic glomerular nephritis; crosses, arteriosclerosis; circles other bilateral destructive kidney diseases, chiefly surgical renal conditions.

of edema; the other six when heart failure had caused the non-protein nitrogen to rise suddenly. Serum phosphate does not seem to be affected as much as nitrogen by either acute renal disease or heart failure. It is for this reason, probably, that in arteriosclerotic nephritis, in which heart failure is more frequent, and in surgical renal conditions high non-protein nitrogen is more frequently seen without hyperphosphatemia or with relatively little elevation of phosphate. This tendency is illustrated in figure 5 by the comparatively large number of crosses and circles that lie above and to the left of the general line of points. It may be this unresponsiveness of phosphate to transitory influences which affect non-protein nitrogen which makes it a more reliable index of ultimate prognosis.

Although in acute nephritis serum phosphate is not regularly increased, even when blood non-protein nitrogen is elevated (60, 140, 244), and seldom rises to the extreme values seen in chronic renal insufficiency, moderate hyperphosphatemia is not uncommon. It is usually associated with azotemia as figure 6 indicates, and occurs in the more severe cases. Values as high as 8 or 9 mgm. per 100 cc. are not, however, incompatible with early and complete recovery (244).

In the nephrotic syndrome serum phosphate usually remains normal (23, 253, 272). At times, however, transitory increases are observed in the absence of renal insufficiency, azotemia or any demonstrable change in the course of the disease. The inexplicable character of these phosphate increases was pointed out by Peters, Wakeman, Eisenman and Lee (253). Because they were found on infrequent occasions in only 2 patients the possibility of technical errors was considered. Further similar observations with controls on additional cases have convinced the author that analytical methods were not at fault. If renal insufficiency develops in the course of nephrosis serum phosphorus rises as it does in chronic nephritis of other kinds with renal insufficiency (244).

The frequent association between hyperphosphatemia and serum alkali deficit led Marriott and Howland (209) to conclude that retention of phosphate was the cause of nephritic acidosis, an opinion which prevailed for some years and is still held by de Wesselow (95, 96). It has, however, become evident from quantitative studies that, although phosphate accumulations undoubtedly contribute to the displacement of bicarbonate, they are seldom large enough to account for more than a small fraction of the alkali deficit (23, 77, 206, 251, 272). Moreover, bicarbonate is often low when phosphate is normal (244, 251, 286).

Hyperphosphatemia has been ascribed to inability of the kidneys to excrete the phosphate ion, although direct and conclusive proof of such inability has not been adduced. The fact that serum phosphate increased after complete ablation of renal function (20, 22) no more constitutes such evidence than does the correlation between serum

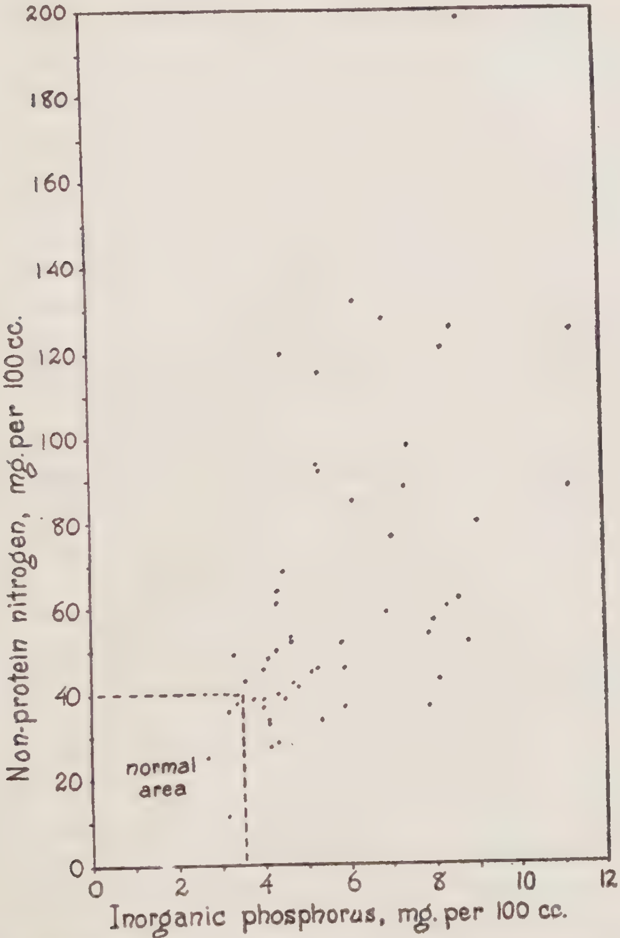

FIG. 6. THE RELATION OF SERUM PHOSPHATE TO BLOOD NON-PROTEIN NITROGEN IN ACUTE NEPHRITIS

phosphate and blood non-protein nitrogen which has already been discussed. Nevertheless these facts, taken as circumstantial evidence, may lend weight to the attitude of those who insist that the burden of proof lies on anyone who would exonerate the kidneys. The problem can be considered only in the light of what is known of the normal mechanism of phosphate excretion. Phosphate belongs among the ions which may be excreted in variable and sometimes considerable part by the bowel, and the partition of phosphorus between stools and urine is dependent upon many purely extrarenal factors (250). Excretory studies that are confined to the examination of urine alone are, therefore, quite inadequate, especially if they are limited to the determination of concentration. Solon (297), Rayer (262) and Frerichs (120) early reported low urinary phosphate concentration in advanced nephritis, Stewart (302) also in the early stages of acute nephritis. Since then low concentration (120) or fixed concentration (63, 98, 216, 289) has been reported by numerous observers. Fleischer (117) found less phosphorus in the urine of chronic interstitial nephritics than in that of normal subjects on similar diets. Fecal excretion was little increased in the nephritics. Kövesi and Roth-Schulz (169) found excretion and retention of phosphorus quite irregular and unrelated to the nature of the nephritic condition. Excretion in the stools was also extremely variable. The largest positive balances accompanied development of edema and could obviously be referred to distribution of phosphate to the edema fluids. Negative phosphorus balances during elimination of edema have been reported by Heinelt and Seidel (147). Others have discovered retarded or incomplete recovery of inorganic phosphate given intravenously or perorally (117, 289). All these studies leave something to be desired because they neglect entirely the condition of the phosphorus stores in the body. Phosphoric acid is not merely a waste product. The organism can, apparently, utilize it in the elaboration of the innumerable inorganic and organic phosphorus compounds, integral and important functional components of all tissues. In a sense phosphate of serum may be quite as well material in transit to points of construction as waste en route to organs of elimination. Phosphorus depletion is not necessarily marked by reduction of serum phosphate. Boyd (60) found phosphorus balances usually negative in nephritis and could establish positive balances only by giving unusually high phosphorus diets. Heinelt and Seidel (147) studied balances in 2 nephritics, one with high serum phosphate. Both had negative balances on low phosphorus diets, positive balances when large amounts were given.

Simultaneous studies of serum and urine phosphate should be of far more significance than studies of urine alone, and have been attempted. Dorner (98) claims that in nephritis the urinary phosphate excretion is low in comparison with the serum phosphate concentration. Heinelt and Seidel (147) in studies over short periods secured inconsistent results. The claims of Dorner and these methods of study are meaningless unless some relation between urinary and serum phosphate levels can be established in normals. Studies of Addis, Meyers and Bayer (5) and others have revealed no semblance of such a relationship. Moreover, urinary and serum phosphate may vary independently under such diverse influences as diuretics of various kinds (54, 55), alterations of carbohydrate or acid-base metabolism (250), or injury to the hypophysis or the tuber cinereum (75). So independent are serum and urine phosphate that Eichholtz, Robison and Brull (102) were tempted to suggest that urinary phosphate was formed in the kidney from organic phosphate esters of blood. This Brain, Kay and Marshall (64) have shown is unlikely.

Addis, Meyers and Bayer (5) found that in rabbits the urinary excretion of phosphate did vary with the serum phosphate after neutral phosphate injections. In nephritis Salvesen (272) and Schultz (289) found that after intravenous or peroral phosphate administration serum phosphate rose higher and returned to the original level more slowly than usual. On the other hand Fetter (112) gave dibasic sodium phosphate to a nephritic with high serum phosphate and alkali deficit. The serum phosphate remained unchanged, but bicarbonate returned to normal and urinary phosphate increased. Fetter is inclined, on the basis of his studies, to the view that the hyperphosphatemia, far from being the cause of the alkali deficit, is a reaction to compensate for the bicarbonate reduction.

It is evident that the cause of the phosphate accumulations is still uncertain and that more extensive controlled studies are required before an unqualified expression of opinion on the subject is warranted. The clinical value of phosphate determinations is, on the contrary, incontrovertible. In chronic renal insufficiency hyperphosphatemia is of grave prognostic significance; normal serum phosphate with azotemia indicates the introduction of some complicating factor such as heart failure or acute infection which may be transitory or amenable to treatment. Little confusion should be occasioned by the occasional high serum phosphate values which occur in patients with the simple nephrotic syndrome.

Brain and Kay (63) have proposed as a test of renal function determination of urinary phosphorus after the intravenous injection of 5 grams of sodium glycerophosphate. They find the excretion greatly impaired in the terminal stages of chronic nephritis, more variable in the arteriosclerotic forms of the disease, little reduced in acute nephritis. These results have no exact bearing on the inorganic phosphate problem, because much of the glycerophosphate is excreted in the organic form. Surprisingly low excretion was observed in 3 out of 6 cases of "nephrosis."

CALCIUM

Low serum calcium is very common in patients with the nephrotic syndrome (3, 137, 166, 249, 272, 273, 292) and is frequently encountered in those with advanced renal insufficiency (33, 60, 66, 95, 96, 166, 172, 232, 249, 260, 272, 273, 286, 289). The reductions in nephrosis appear to be clearly related to the serum protein deficits. Marrack and Thacker (208), Loeb (192, 193, 196) and Hastings, Murray and Sendroy (143) have all shown that proteins increase the solubility of calcium in true and artificial sera, probably by the formation of non-ionized or only slightly ionized Ca-protein complexes or compounds. Formulae for the quantitative evaluation of the calcium held in solution by protein have been proposed by Hastings, Murray and Sendroy (143) and by Peters and Eiserson (249). Greenwald (135), on the basis of some other data, has questioned the accuracy of these formulae and proposed a new one without, however, questioning the fact that there is a direct relation, probably linear, between the concentrations of protein and calcium in sera. Hypocalcemia in conjunction with serum protein deficiencies has been noted in conditions of malnutrition (153, 244) and has been produced in dogs by plasmapheresis (88). The ability of protein to carry calcium probably explains the low calcium content of serous transudates and lymph (20, 133, 143). The difference between the calcium concentrations of serum and transudates, taken simultaneously from the same subject, are maintained when the two fluids are dialyzed against one another with collodion membranes through which serum calcium, under other conditions, appears to be freely diffusible (194). Arnold and Mendel (20) found that procedures which altered serum calcium had an equal effect on lymph calcium, but did not change significantly the difference between the calcium concentrations in the two fluids. All these experiments afford a rational explanation of the serum calcium deficiencies of nephrosis. They also indicate that the calcium combined with protein, of which a part is lost when the serum proteins fall, being little ionized and confined to the blood stream by its non-diffusibility, can play little or no part in processes of calcification or other general functions of calcium. The infrequency of tetany and disorders of calcification, in spite of low serum calcium, in patients with the nephrotic syndrome is probably due to the physiological inactivity of this protein fraction.

The reductions in advanced renal insufficiency can be only partly accounted for by serum protein losses. The calcium deficiencies may occur when proteins are normal or may be extreme when proteins are only slightly diminished (244). Salvesen and Linder (273), in 1923, concluded that low serum calcium was found in nephritic patients only when serum protein was low or when serum inorganic phosphate was high, an observation that has found ample confirmation (3, 60, 95, 96, 168, 208, 249, 272, 286, 288, 292). Marriott and Howland (209) had earlier suggested that retention of phosphate was responsible for the serum calcium deficiencies. There are excellent theoretical reasons, supported by much experimental evidence (cited in 249 and 250), for believing that the concentrations of inorganic phosphate and calcium in the serum are reciprocally related. For this reason Peters and Eiserson (249) attempted to estimate the quantitative effect of phosphate on calcium by statistical analysis of data secured from nephritic patients. There was a demonstrable correlation between the two variables, although this was not nearly as satisfactory as that between protein and calcium. The unsatisfactory nature of the correlation the authors ascribed partly to associated disturbances in the pH and electrolyte concentrations in the sera, which were more common and greater in the cases with the largest phosphate accumulations. In general the greatest discrepancies were observed in terminal stages of the disease when inorganic phosphorus was extremely high. Greenwald (135), in discussing the formula of Peters and Eiserson, denies that there is any reciprocal relation between calcium and phosphate, a rather extreme position in view of the evidence cited above. Accumulation of further data has not led the author to alter essentially his opinion that the hypocalcemia of advanced renal insufficiency is connected with the hyperphosphatemia with which it is invariably associated. The inaccuracy of the earlier attempt to quantitate the relation between calcium and phosphorus, especially by means of data secured from nephritic subjects with profound disturbances of acid-base and electrolyte equilibria, has become more and more apparent. This material was selected to escape the confusing influence of conditions which involve disturbances of parathyroid activity or calcification. It is, however, quite uncertain whether nephritic patients are free from such disturbances.

Bennett (33), de Wesselow (95) and others have attributed the convulsions and other signs of motor hyperactivity of "uremia" to the serum calcium deficiency, by implication identifying them with tetany. That tetany may develop after phosphate administration when serum calcium falls below a certain level was demonstrated by Binger (35). In the same experiments it was shown that alkaline phosphate mixtures were more effective in provoking tetany than were acid phosphates. More recently acid ammonium phosphate has been used successfully for the treatment of spasmophilia (256). Experimental and clinical observations together seem to indicate that tetany results from either reduction of serum calcium or displacement of the serum pH in an alkaline direction. The effects of calcium deficiency may be combatted by acidifying measures (250). Increase of serum phosphate *per se* seems to be without influence. In nephritis serum calcium seldom falls to the levels which induce tetany, unless there is a hypoproteinemia. In the latter case the deficiency involves in whole or in part the fraction of calcium which is combined with protein. This, it has been pointed out, appears to be physiologically inactive and devoid of antitetanic powers. Furthermore, in advanced renal insufficiency, when the hypocalcemia is referable to phosphate alone, there is invariably an acidosis which tends to combat the tetanogenic effect of the calcium deficiency. Muscular hyperactivity and convulsions do not, in themselves, denote tetany or the spasmophilic tendency. This is associated with definite abnormalities of electrical excitability and certain characteristic signs. Schmitz, Rohdenberg and Myers (286) and Nelken and Steinitz (232) were unable to detect any exact correlation between serum calcium concentrations and either muscular twitchings or convulsions. A limited number of observations made by members of the author's staff revealed normal electrical reactions and absence of Chvostek's and Trousseau's signs in uremia, in one case even when serum calcium was only 5 mgm. per 100 cc. Serum protein and bicarbonate were both moderately reduced, phosphate considerably increased, 9 mgm. per 100 cc. Finally, both convulsions and muscular twitchings are seen in patients without any considerable disturbances of either phosphate or calcium of the serum.

Tetany may occur in characteristic form in nephritis and is quite easily distinguished from ordinary uremic manifestations. Usually it results from vomiting, overventilation, or over zealous bicarbonate therapy (104) and is, therefore, referable to alkalosis, not to calcium deficiency. However, if serum calcium is low, the influence of alkalosis would presumably be exaggerated. In 1 case in the author's series with persistent vomiting, outspoken tetany with convulsions occurred. This was referred to the vomiting, especially when serum chlorides were found to be greatly reduced. When parenteral sodium chloride proved ineffectual, serum calcium was determined and proved to be 4.0 mgm. per 100 cc., although protein was within normal limits and phosphate only moderately elevated, 7.7 mgm. per 100 cc. In this instance some additional factor, not usually active in nephritis, seems to have determined an excessive reduction of serum calcium. Klinke (165) has reported a case of nephrosis with recurrent tetany associated with low serum calcium. These sporadic cases can only be explained when more is known of the effect of nephritis on calcium metabolism. It will be pointed out later that the disease may be associated with extreme calcium wastage and decalcification of bones. Mainzer (201) has reported 3 cases with outspoken tetany for which no explanation could be found in serum calcium or bicarbonate. Mainzer believes, because phosphate was high in all, that they demonstrated a specific spasmophilic effect of phosphate, an opinion that is at variance with physiological studies.

Studies of calcium metabolism in nephritis are extremely scanty. Boyd (60), Scriver (292) and Albright and Bauer (10) have found urinary calcium low in nephrosis. Hetényi and von Nógrádi (151) claim that intravenously injected calcium is excreted more slowly and less completely by nephritics than by normals. Calcium balances in all types of nephritis Boyd (60) found extremely variable, more often negative than positive. He was unable to detect any clear relation between the excretion of calcium and its concentration in serum. Fecal excretion of calcium was often abnormally high and the faulty absorption of calcium seemed to be connected with high serum phosphate concentrations. Schoenthal and Burpee (288) found excessive fecal calcium losses in a child with advanced renal rickets, high serum phosphate and low serum calcium. It may be of interest to note, in relation to the calcium excretion in nephrosis, that Jansen (153) has reported large fecal losses of calcium in patients with malnutrition edema who were receiving diets containing adequate amounts of both calcium and phosphorus. Although there is little accurate knowledge of calcium and phosphorus metabolism in nephritis, there is some evidence that calcium wastage may occur in the disease.

Apparently because of the low serum calcium, parathyroid hormone has been used and recommended for the treatment of nephrosis (80, 188, 213, 215, 265). The results of this therapy have been variable and are difficult to evaluate. As a diuretic parathormone is only irregularly effective. In two cases the author has seen no benefit from its use. No clear relation has been established between the tendency to edema and calcium metabolism, nor is there any good theoretical ground for believing that reduction of the protein fraction of serum calcium is related to parathyroid deficiency. In Scriver's (292) cases parathormone increased neither serum calcium nor urine calcium, although fecal loss was augmented. Scriver suggests that nephrosis is associated with parathyroid deficiency and impairment of urinary calcium excretion. However, Albright and Bauer (10) were able, in their case, with ammonium chloride, to sweep both calcium and phosphorus into the urine without altering serum phosphorus or serum calcium. Further investigation is needed to establish the therapeutic value and the mode of action of parathyroid hormone in the treatment of nephritic edema.

The diuretic effect of calcium chloride, which was first ascribed to the specific action of the calcium ion (43, 266), is now known to depend upon the acidifying properties of the salt (24, 123).

RENAL DWARFISM AND RICKETS

The incidence in long standing renal disease, especially among infants and children, of skeletal abnormalities, seems to have been noted first by Goodhart (127) and has come to be recognized as a regular result of this condition (26, 100, 132, 136, 149, 152, 157, 170, 179, 221, 239, 241, 288, 291, 298, 326). The skeletal abnormalities are of two types: (*a*) deficient growth which gives rise to infantilism or dwarfism and (*b*) osteoporosis or decalcification of the bones, "renal rickets." Chief interest, from the metabolic point of view, centers about the latter and its identification with infantile rickets. From the morphological view there has been considerable controversy which has been reviewed by Mitchell (221), Schoenthal and Burpee (288), Greene (132), Paterson (241) and others. The conflict has not been lessened by the inclusion, among the records, of infants with outspoken rickets. When the condition appears in early infancy it is hard to decide whether it is due directly to the renal pathology or whether the latter, by interfering with health, appetite, digestion and nutrition has only acted as a contributory factor by rendering the subject more susceptible to rickets or interfering with the ingestion or absorption of antirachitic vitamin. In older children, on the other hand, the more mature character of the epiphyses may account for the absence or distortion of the pathological changes which, in the infant, are considered characteristic of rickets. Osteoporosis and absorption of bone, affecting both epiphyses and shafts, undoubtedly result in patients who have suffered from long-standing renal insufficiency, regardless of the nature of the kidney disease, especially during the period of growth and development.

The serum electrolyte picture in such patients exhibits the disturbances already described as characteristic of advanced renal insufficiency. Because of the bone changes especial emphasis has been laid on calcium and inorganic phosphate determinations. Phosphate is usually high, calcium often low (100, 136, 152, 157, 170, 179, 288, 291, 298). For this reason the osteoporosis has been identified by some with low calcium high phosphate rickets. There is, however, no reason for believing that hyperphosphatemia in itself can cause rickets or osteoporosis. In fact increase of serum phosphate with simultaneous diminution of calcium is commonly observed in the early stages of healing of infantile rickets. Howland and Kramer early pointed out that neither calcium nor phosphorus alone determined the appearance of decalcification or healing, but the product of the two. In the majority of reported cases of renal rickets calcium-phosphorus products are normal; sometimes they are even high (100, 152, 298). Similar products in non-nephritic subjects would be considered conclusive evidence of the absence of rickets. For this reason Schoenthal and Burpee (288), Paterson (241) and others believe that the osteoporosis must be attributed to some other factor. The former demonstrated constant wastage of calcium and phosphorus in the stools of one subject with renal rickets and high serum phosphate.

Among the disturbances most consistently found in advanced renal insufficiency and in all the cases of renal rickets in which adequate studies have been reported, is acidosis. Serum bicarbonate is consistently low (100, 136, 179, 288). It has been demonstrated repeatedly and conclusively that acidifying measures or conditions invariably increase the elimination of calcium and phosphorus, which are abstracted from the bones to aid in the neutralization and excretion of the excess acid. So potent is the decalcifying action of acidifying measures that they will sweep out calcium and phosphorus even after the effect of parathormone has been exhausted (11). It seems not unreasonable to infer that the osteoporosis and, perhaps, some of the serum calcium deficiency of advanced renal disease is due to the decalcifying effects of prolonged acidosis, an explanation that has already been advanced (136, 288). The author has, in one instance, observed osteoporosis in an adult with long standing alkali deficit associated with destructive renal disease. For eight years, while she was under observation, serum chloride was greatly elevated, while bicarbonate remained continuously low. Attempts to reduce serum chloride by salt restriction resulted, as usual, only in simultaneous reduction of chloride and base, without affecting bicarbonate. Administration of bicarbonate, although effective in restoring the normal acid-base equilibrium and chloride-bicarbonate partition of the serum, provoked epileptic convulsions, to which the patient was subject, and therefore had to be discontinued. Throughout the period of observation serum calcium remained low, although protein was almost normal and phosphate but little reduced. In this case a spontaneous chloride acidosis seems to have exerted the same effect on the bones as a similar acidosis caused by the administration of ammonium chloride.

Whether one wishes to call the osteoporosis of long standing renal insufficiency rickets or not would seem to be merely a matter of opinion and definition. It seems to be more consonant with present conceptions to confine the term rickets to conditions resulting from lack of vitamin D. György (136) in one case, a child of ten who had suffered from an infection of both kidneys for four years and presented retarded growth, deformities, spontaneous fractures, osteoporosis and rachitic changes, secured clinical improvement by the administration of vigantol. The child had low serum calcium, high phosphorus, a high calcium-phosphorus product, and the usual bicarbonate deficiency. The improvement under antirachitic therapy György adduces as evidence that the bone condition was rickets. This deduction seems hardly warranted. The action of irradiated ergosterol in promoting absorption and storage of calcium and phosphorus is not confined to rickets. In the author's case, described above, irradiated ergosterol and calcium seemed to check the osteoporosis and to increase serum calcium. Schoenthal and Burpee (288) in their case were able to check calcium wastage by a high calcium diet with an alkaline ash.

CONCLUSIONS

The chief conclusions that can be drawn from the present imperfect knowledge of salt and water metabolism in nephritis are: (*a*) that many of the disturbances to which especial significance has been attached are only remotely related to the pathological changes in the kidneys; (*b*) that they are more often results than causes of symptoms. Expansion of our understanding of these disturbances requires the abandonment of certain unphysiological preconceptions and catch words, such as "renal permeability," and the inclusion in consideration of nephritic problems of the whole body of information concerning the forces which may dislocate or readjust the internal environment. Maintenance of a normal internal environment would seem and has proved to be conducive to comfort and well-being in nephritis as in health; but as the exclusive aim of treatment is quite inadequate, if not dangerous. Especially is this the case if attention is given only to the concentration of various solutes in blood or serum. Determinations of the concentration of any or all solutes in available body fluids gives but a partial impression of the state of the organism with respect to these solutes. From the preceding discussion it must be evident that, for example, the serum chloride concentration may be high in the face of a general chloride deficiency which is masked by dehydration and attendant hemoconcentration. Salt restriction, in this case, even if it should restore a normal electrolyte pattern, would represent misdirected effort, because it could only exaggerate chloride depletion. In the same manner, bicarbonate administration is not the best universal measure for combatting serum bicarbonate deficits. The general inference that accumulation of any substance in the blood is directly referable to inability of the kidneys to excrete that substance has no substantial foundation in physiology and has given rise to many erroneous conclusions and misdirected therapeutic efforts. More unfortunate is the inhibitory influence which the satisfying nature of such inferences has had on clinical investigation. Undoubtedly some of the distortions in the composition of the blood are expressions of compensatory reactions. The adjustive reactions of the nephritic organism differ quantitatively, rather than qualitatively, from those of normal subjects. Obviously protective reactions must be recognized in order that they may be supported, not resisted. Mere restoration of a normal electrolyte pattern may be of doubtful value if the underlying functional defect which produced the original disturbance of serum composition can not be eliminated. In fact well reasoned attempts to distort patterns, illustrated by the use of acidifying diuretic measures, may be of the greatest benefit.

Finally it is to be emphasized that in renal diseases, as in other pathologic conditions, chemistry gives information concerning function rather than structure. Imperfect, but ever better, impressions of structural alterations can be gained, with increasing knowledge, by the proper critical evaluation of functional changes. However, chemistry is, and will probably remain, less important as a diagnostic aid than as a therapeutic guide.

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